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UNIVERSITY OF ARKANSAS

COLLEGE OF AGRICULTURE

Agricultural Experiment Station

A BACTERIAL DISEASE OF FOXTAIL
(CHAETOCHLOA LUTESCENS)

BULLETIN NO. 193

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A BACTERIAL DISEASE OF FOXTAIL (CHAETOCHLOA LUTESCENS)[1]

H. R. ROSEN

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Introduction

In a brief note published in March, 1919, the writer called attention to a disease of foxtail common in Arkansas. It was pointed out that the pathogen, a whitish bacillus, was capable of attacking a number of grasses, including most of the common cereals. Since then an intensive study has been made of this disease, with the object of establishing its symptoms and etiology, and in the pursuit of this investigation facts were evolved which clearly showed the necessity of using certain methods in the study of plant pathogens which are not ordinarily in vogue among plant pathologists.

Bacterial Diseases of Gramineae in General

Since the studies here presented indicate that the disease producer has not been previously described it seems desirable, for comparison, to review briefly the bacterial diseases of the *Gramineae*. Smith (I, '20, p. 7)[2] has recently given a list of plant genera which are known to be attacked by one or more bacterial species. In this is included the following grass genera: *Hordeum, Dactylis, Bromus, Zea, Setaria, Andropogon, Avena, Saccharum, Secale, Triticum, Phleum, Poa*, and *Agropyron*. These 13 genera, he also states (p. 4), are attacked by 14 different disease producers. Not having mentioned these by name or by citation to literature, it becomes rather difficult to ascertain the organisms which he included. It is quite possible that 1 or more of these 14 have not as yet appeared in print and are known only to Dr. Smith. Of the host genera which he lists, no reference has been found to a bacterial disease of *Bromus*,[3] and aside from Manns' (I, '09) brief reference to a disease on the blue grasses which, according to the author, appeared to be the same disease as that on oats, no other literature dealing with a bacterial disease of *Poa* has

[1]Reprinted from Annals of The Missouri Botanical Garden, 9: 333-402. Nov. 1922.

[2]The bibliography is divided into two parts, the first part dealing with references to bacterial diseases of grasses, designated I, and the second part dealing with morphological and physiological references, designated II.

[3]Since this work was written and published in the Annals of The Missouri Botanical Garden, Chas. S. Reddy and James Godkin have presented a paper on "A Bacterial Disease of Brome-grass" (Phytopath. 13: 74-86. 1923). They consider the pathogen very closely related to *Bacterium coronafaciens* Elliott but because of the difference in susceptibility of different grasses, it is considered as a different variety *(atropurpureum)*.

been found. Concerning a bacterial disease of *Phleum* but little more information is available. Manns refers to a bacterial disease of timothy in the same sentence, cited above, in which reference is also made to a disease of *Poa*. In addition to this reference, a blade blight of timothy "apparently bacterial" was reported in Ohio during 1918 and 1919 by the Plant Disease Survey (I, '19, p. 157, and '20, p. 76), and Jones, Johnson, and Reddy (I, '17) mention a bacterial disease of *Phleum pratense*.

Of the bacterial diseases of grasses which are well known or supposedly well known it will perhaps be desirable to characterize briefly each one, noting the similarities and the differences as compared with the foxtail organism. A chronological sequence will be followed.

One of the very first investigators of bacteria in relation to plant disease and perhaps the first to study carefully a bacterial disease of a grass was Prillieux. His account of a rose-red disease of wheat kernels (I, '78) is well known. He made no pure culture studies, so that it is not possible to identify definitely the organism he described. Gentner (I, '20) has recently published an account of a bacterial disease of barley in which he states that the pathogen when inoculated in pure culture into sterilized kernels of barley, wheat, and corn produces a reddish discoloration. He believes that the organism with which he worked, a rod-shaped, red-pigment producer, named by him *Bacillus cerealium*, is the same as Prillieux's organism and that the *Micrococcus* which Prillieux found associated with discolored wheat kernels represents the spore stage of *B. cerealium*. The fact that this organism produces a red pigment on various media and is a spore producer, an anomaly among plant pathogens, would immediately distinguish it from the foxtail organism.

In 1887 Burrill described a disease of broom-corn and sorghum which he attributed to bacteria. Two years later, Kellerman and Swingle (I, '89), under the name of "sorghum blight," published an account of a disease which they considered the same as Burrill's; they also accepted and seemingly substantiated his work on the pathogenicity of the organism which he had named *Bacillus Sorghi*. Several other investigators before and after Burrill's time have worked on red spots of sorghum, but to this day we have little exact information on the disease. Thus Palmerie and Comes in 1883 decided that red discolorations of sorghum were due to yeasts and bacteria; Radais in 1899 concluded that sorghum blight was due to yeasts; and Busse (I, '05), in a rather extensive paper, showed that various causes can produce reddening of sorghum. Concerning any bacterial disease of sorghum, Busse concluded that there was no specific bacterial pathogen involved, that the bacteria which have been found within the tissues were merely faculative parasites which entered by means of injuries induced by insects and other agents

and that *Bacillus Sorghi* was an organism of this sort. The writer does not intend to go extensively into this disease, or rather group of diseases, at this time; suffice it to say that as far as the written descriptions of *B. Sorghi* are concerned the organism cannot be identified. While apparently accepting Burrill's organism in an early publication (Smith and Hedges, I, '05) Smith handles it with great caution in other publications (I, '05, p. 66, p. 92, pl. 20 [opposite p. 150]; '11, p. 62 and 63), not definitely rejecting it but substituting another name, *Bacterium Andropogoni*. Smith's complete account of *B. Andropogoni* follows: "It is non-sporiferous, polar-flagellate (1-3), and white on culture media, forming small circular colonies on agar-poured plates. It is aerobic, non-liquefying, non-reducing (nitrates)." Manifestly this preliminary description also is so incomplete that it is impossible to make any adequate comparison with an unidentified organism. We may note, however, that the foxtail organism possesses similar characters with the exception that it has but a single polar flagellum and is a marked nitrate reducer. The writer's preliminary studies on a bacterial disease of sorghum present in Arkansas indicates that the pathogen there concerned is different from the foxtail organism.

Following the description of a bacterial disease of sorghum Burrill (I, '89) described a bacterial disease of field corn. For the present I may merely refer to my descriptions of a bacterial disease of field corn (I, '19[a], '21) and to note, as will be fully discussed in another publication, that Burrill's organism is either not identifiable or is not related to disease production. The pathogen is quite unlike the foxtail organism.

In 1893 Cobb described a bacterial disease of sugar cane. The disease is so thoroughly described by Smith (I, '14, pp. 3-71) that there is no necessity for discussion of it here. We may note, however, that *Pseudomonas vascularum*, a yellow rod, is typically a vascular disease producer. These characters alone are sufficient to distinguish it from the foxtail organism.

A bacterial disease of sweet corn was first described by Stewart (I, '97); this, like the sugar cane disease, is also due to a vascular parasite, which is yellow in color, and according to recent investigations (McCulloch I, '18), is non-motile. All these features, as well as others, indicate that it is quite distinct from the foxtail pathogen.

Another non-motile, yellow parasite is *Aplanobacter Rathayi*. It was first described by Rathay in 1899 as attacking orchard grass near Vienna. According to Smith (I, '14, p. 155), who gives a good account of this disease, it is also present in Denmark. Descriptions indicate that this also is distinct from the motile, white, foxtail organism.

In 1901 Guffroy described knots on rhizomes of *Arrhenatherum elatius* which he decided were brought about by bacteria; he

named the organism *Bacterium moniliformans*. The original article has not been seen by the writer, but according to Sorauer (I, '08, p. 30) Guffroy has presented no proof for his conclusions.

Voglino (I, '05, pp. 43-44) described a bacterial disease of rice, present in Italy. The large size of the organism, 2.5–3.5 × 3.8–4.0 μ, should readily distinguish it from all other organisms attacking grasses.

Manns (I, '09) published an extensive account of a bacterial disease of oats, although Galloway and Southworth as early as 1890 and Russell in 1892 briefly described a bacterial disease of the same host. It may be noted that Manns' conclusions concerning the cause of the disease have been questioned, and more recently Miss Elliott (I, '20) has given a detailed account of a halo-blight of oats. Concerning the disease which Manns described, she says (p. 167); "His colored figures as well as most of his text indicate an entirely different disease, but his Plate XIII shows that this halo-disease formed at least a part of the phenomenon under consideration." She states further (p. 168), that "Manns' descriptions of individual lesions are so meager and his descriptions of general signs so inclusive as to lead to grave doubt about his having worked with a single bacterial disease." This statement is somewhat different from the one made by Miss Elliott in a previous publication (I, '18). In this she says, "The characteristic halo lesions of this disease have been definitely observed in Wisconsin during each of the past five years and are similar to those figured by Manns (Pl. XIII) in his Ohio bulletin of 1909. The results of the writer's work on halo blight of oats agree with those reported by Jones, Johnson, and Reddy* ('17) in that typical halo lesions are readily produced by a white bacterial pathogen alone." In view of the fact that Manns had included a number of grasses as hosts and that Jones and his associates had accepted *Pseudomonas Avenae* as the pathogen I made the following statement in a preliminary note on the foxtail disease: "The effect on oats is not unlike the halo blight recently described by Miss Elliott and it is likely that the organism under discussion is the same as Manns' *Pseudomonas Avenae*. However the identity of the organism is still in doubt and the work is being continued."

The writer is now in a position to say that Manns' description of *P. Avenae* is quite at variance with the account of the foxtail organism which will be presented later. It must be borne in mind, however, that bacteriological methods and descriptions in 1909 were not as well developed as they have become in more recent years, and when, for example, endospores are recorded in 1909 it must not be assumed that this is an established fact; the same thing may be said for a large number of cul-

*These writers considered Manns' *Pseudomonas Avenae* as the causal organism.

tural reactions. However, while the foxtail organism may be present in Ohio, Manns' description as well as Miss Elliott's, even when interpreted very broadly, clearly indicates a difference in the organism. A comparison of group numbers, using the 1907 chart of the American Bacteriological Society, shows at a glance the marked difference between the foxtail organism and *P. Avenae*. Manns' group number (I, '09, p. 133) is 111.2223032, while the group number of the foxtail organism is 212.3333013. Among other differences it may be noted that the foxtail organism does not liquefy gelatin, produces no acid, even in the presence of carbohydrates, and produces strong diastatic action, characters quite different from those described for *P. Avenae*.

Miss Elliott was kind enough to send the writer a culture of *Bacterium coronafaciens*, the causal agent of the halo-blight of oats, and comparison of this organism with the foxtail pathogen shows distinct differences. As Miss Elliott states, *B. coronafaciens* usually occurs in chains and produces a brownish color on nutrient agar; these characters serve to distinguish it immediately from the foxtail organism, which usually occurs singly or in pairs and produces no brown color on nutrient agar. There are also a number of other morphological and physiological differences which can be readily ascertained by comparing the description of the foxtail organism given below with the one given by Miss Elliott (I, '20). Before closing the reference to bacterial diseases of oats the writer wishes to call attention to a pathogen isolated by him from reddish spots of oats, an organism which when inoculated in pure culture, produced the same kind of spots (no halos are produced). This organism, a white, monotrichous rod, is a strong gelatin liquefier and does not attack foxtail. It apparently is not *Bacterium coronafaciens*.

A bacterial disease of western wheat-grass was described by O'Gara in 1915 (I, '15, '15[a]). The pathogen, *Aplanobacter Agropyri*, is yellow and non-motile and thus quite different from the foxtail organism.

In 1916 Jones, Johnson, and Reddy briefly described a bacterial blight of barley and certain other cereals. This was followed by a more complete description in 1917. In this, also, a yellow organism, *Bacterium translucens*, is involved. Besides barley, these writers have found that wheat, spelt, rye, timothy, and oats are attacked by similar bacterial diseases, but that the barley organism, *B. translucens*, does not attack the other grasses listed above. Attention has already been called to a bacterial disease of barley found in Germany by Gentner (I, '20). While proof has been given that *Bacillus cerealium* may produce disintegration of seed, it seems to me that more work would be necessary before any conclusion could be drawn regarding the production of brown spots on various organs of the barley plant. As indicated previously the organism differs greatly from the foxtail pathogen.

A new disease of wheat, now well known as black chaff, was first described by Smith in 1917. The pathogen is closely related to the one causing barley blight and has been named by Smith, Jones, and Reddy (I, '19) *Bacterium translucens* var. *undulosum*. This organism goes as readily to barley as the barley organism itself, while the barley organism hardly attacks wheat. It also is a yellow pathogen.

Another bacterial disease of wheat was found in India by Hutchinson and first described in 1917. The descriptions indicate that the disease is quite similar to the one described by O'Gara (I, '15, '15[a]) on western wheat-grass. There is a marked distortion of the heads and the spikelets are covered by a yellow slime. The pathogenicity of the organism, *Pseudomonas Tritici* Hutch., has not as yet been definitely established. The symptoms, particularly the yellow slime, would indicate that it is quite different from the foxtail disease.

For convenience a third bacterial disease of wheat, the basal glumerot, may be considered next. It was discussed by Miss McCulloch in 1920, and the pathogen, *Bacterium atrofaciens*, described as a greenish pigment producer, is quite distinct from the foxtail organism.

In closing the historical resumé of bacterial diseases of grasses it should be noted that no attempt has been made to include all references, but rather those of significance in relation to the disease producer here investigated. The 14 grass genera listed by Smith (I, '20) have been included and besides these reference has been made to a bacterial disease of *Arrhenatherum* and of *Oryza*. In addition special attention may be called to the very brief notice of a bacterial disease of millet (listed under *Panicum* sp.[1] but perhaps intended to be *Chaetochloa* (*Setaria*) sp.) in a supplement bulletin of the Plant Disease Survey (I, '19, p. 157). The note reads: "Blight, apparently of bacterial cause, was noticed on three varieties of millet in Minnesota about August 17." This reference is of particular interest, since millet, *Chaetochloa italica* (*Setaria italica*), is closely related to foxtail, *Chaetochloa lutescens* (*Setaria glauca*) and in the seedling stage is as readily attacked by the foxtail organism as foxtail itself. Various other references have been found to bacterial diseases of grasses, including those which produced spots on rye and on corn, but these are so brief as to be of no special significance for present purposes.

General Description of the Disease

The symptoms of the disease vary somewhat with host and

[1]There are a number of different grasses going under the name of millet, but the names foxtail, millet, or Italian millet are applied to *Chaetochloa italica*, while proso or broom-corn millet are applied to *Panicum miliaceum*. The latter is but sparingly grown in this country. (See Hitchcock, U. S. Dept. Agr., Bur. Pl. Ind. Bul. 772. 1920.)

considerably with conditions under which infection takes place. The pathogen grows best at relatively high temperatures, so that other conditions being favorable, at a temperature of around 90° F. the first signs of infection will occur in 24 to 48 hours. Under very moist conditions the spots appear water-soaked at first and as the atmosphere becomes dry they appear as light brown or grayish brown spots or streaks. On foxtail and on *Chaetochloa geniculata* these spots may be bordered by a distinct brown or reddish brown area (see pl. 1, fig. 1). Under favorable conditions of infection attacked areas often appear grayish green, withered, looking as if scalded, and surrounded by a somewhat indefinite yellowish halo. The spots on foxtail as they occur naturally in the field, in a dry atmosphere, are usually of two types, (1), small, reddish brown, round or oval-shaped areas enclosing lighter brown centers, and (2), light or dark brown or blackish streaks, usually starting at the tip of the leaf and often including a large part of leaf area. The spots may appear on any part above ground, including leaf-sheath as well as blade, rachis, and glume. Under natural conditions spots are more often to be observed on the leaf blades.

On oats the spots vary from light yellow, somewhat indefinite areas to grayish green, markedly withered areas. Often there are marked tinges of red[1] in attacked areas, particularly when a number of infections have coalesced. Attacks may occur on seedlings or on plants that are in head. Numerous artificial inoculations have been made on various varieties of oat seedlings, and under favorable conditions of infection not a single oat plant out of a large number inoculated would remain alive 4 days after inoculation (see pl. 4, fig. 1). It is of course evident that a seedling with a limited surface is much more readily killed than a larger plant.

Attacks on wheat, rye, barley, and corn do not vary considerably from that on oats. There is usually very little red discoloration, the spots vary in size and shape, and the predominant discolorations are grayish green, light yellow, or brownish, withered areas. On barley and on corn very light yellow or whitish streaks are not uncommon. Barley seedlings, as well as oats, succumb very readily (see pl. 4, fig. 2).

On millet the attack is usually in the form of grayish green, withered areas, occasionally with brown tinges. On various varieties of sorghum the spots are often grayish green bordered by a red band or merely a small red, irregular spot; reddening, it may be noted, being a much more general character of various sorghum diseases.

[1]The writer is satisfied that various agents can cause reddening of oat leaves. Under certain conditions of light and temperature a mere sharp crease in an oat blade may produce a reddening in the part above the crease. It is well known that the production of anthocyans may take place at low temperatures and when the transfer of foods is cut off, resulting in an accumulation of sugars. Attacks by aphids often appear as yellow or red discolorations.

There are two diseases of foxtail which are of common occurrence in Arkansas as well as in other states, notably Delaware, Missouri, New York, and Pennsylvania, which may be mistaken for the bacterial disease. One of these is caused by *Cercospora Setariae* Atk. and the other is a *Piricularia* spot. The *Cercospora* attack is usually in the form of elongated dark brown streaks which usually exhibit the spore groups and thus are easily identified, while the *Piricularia* spot is usually roundish, light brown in color with a darker brown edge. These *Piricularia* spots may readily be confused with the bacterial spot because of their close resemblance and because the spores are often difficult to find. Microscopic examination will, however, usually bring out the difference. The bacterial spots are full of bacteria which in a water mount with slight pressure will stream out of the tissues in the form of dense, grayish white clouds. They may also be detected within the cells. Rarely are bacterial spots contaminated by fungi.

INTERNAL APPEARANCE AND PATHS OF INFECTION

Sections of diseased leaves show the bacteria present in great numbers within the cells and in the intercellular spaces (see pl. 5, fig. 1). The parenchyma is apparently the only part attacked, since no bacteria have been observed within the bundles. The attacked cells lose their turgidity, suffer partial or complete collapse, are more or less disintegrated, and variously discolored. Externally the collapse of cells is indicated by a shrinking and withering and finally by splits or breaks in the diseased parts. Ordinarily there is no oozing outward of bacteria, but under very moist conditions and where rifts have occurred in the tissues the bacteria are present in great numbers in the drops hanging to the rifts. This undoubtedly leads to the dissemination of the pathogen by rain and by winds. (Spreading by insects is also to be expected.)

Artificial inoculations clearly indicate that the pathogen enters by means of the natural openings, the stomata and the water-pores. Material fixed in chrom-acetic acid, imbedded in paraffin, sectioned, and stained with carbol-fuchsin has shown substomatal cavities full of bacteria, which in later stages of infection are surrounded by cells and intercellular spaces which are also full of bacteria (pl. 5, fig. 1). Regarding infection through water-pores it was noted that under certain conditions in the greenhouse, which included an extreme dryness of the atmosphere, resulting in an incipient wilting accompanied by a closing of the stomata, the few infections obtained occurred at the tips of the leaves, indicating entrance by means of the apical hydathode. This is of interest since it indicates that under natural conditions when stomata are closed, as at night, infection may take place through the water-pores.

HOSTS AND EXTENT OF INJURY

Thus far, foxtail is the only grass that has been found infected under natural conditions. This statement may not be of much significance, as the time spent in looking for the disease on other hosts was, by force of circumstance, very limited. The ease with which artificial infections are obtained on a number of wild and cultivated grasses indicates that the pathogen may be looked for on all the common cereals, particularly in the southern states. The following species representing 5 different tribes are susceptible, as proved by artificial inoculations: *Avena sativa*, *Chaetochloa geniculata*, *Chaetochloa italica*, *Chaetochloa lutescens*, *Holcus Sorghum* (*Andropogon Sorghum*), *Holcus Sorghum sudanensis*, *Hordeum vulgare*, *Secale cereale*, *Triticum sativum*, and *Zea Mays*.

A sufficient number of artificial inoculations and of reisolations have not been conducted with red top (*Agrostis palustris*) and with goose-grass (*Eleusine indica*) to warrant any definite statement, but preliminary tests would seem to show that these also are susceptible.

Artificial inoculations have given negative results with the following: *Poa pratensis*, *Syntherisma sanguinalis*, *Chaetochloa viridis*, *Festuca elatior*, *Oryza sativa*, and *Phleum pratense*.

The disease on foxtail has now been observed in northwest Arkansas for four successive seasons. Specimens have also been obtained from other portions of the state, indicating that the disease is widespread throughout Arkansas. No effort has so far been made to locate the disease in other states, so that little is known concerning its distribution. It should be looked for particularly in the warmer sections of the country, since physiological studies indicate that the organism grows best at relatively high temperatures. Field infections on foxtail are at times severe, especially during warm, moist weather. Under such conditions it is not uncommon to find individual plants with almost every leaf-blade killed and with only the rachis and head showing a normal, green color. Lower leaves in particular are to be found diseased (indicating infections by spattering of rain drops). However, considering the extent of injury as observed over a period of several years there is no reason for viewing this disease with alarm. On one particular field where it has been under close observation for more than three years, the disease has occurred on 4 successive crops of foxtail without any apparent diminution in the number of volunteer plants that sprang up. Every year this field had been plowed, cultivated, and used for growing tomatoes and other crops, and by late summer, unless considerable hoeing were done, foxtail would "take" the field. In this connection it is worth while calling attention to the distribution and growing season of the host. Yellow foxtail is common in cultivated soil in the eastern United States (it is also very

common in the Mississippi region) according to Hitchcock (U. S. Dept. Agr. Bull. 772, p. 243) and is "often sufficiently abundant to furnish considerable forage." It usually does not appear before midsummer and comes into full development by late August or early September. Thus we see a high, midsummer temperature favoring host development as well as that of parasite.

SUSCEPTIBLE VARIETIES OF COMMON CEREALS

The following varieties of common cereals[1] have been found susceptible in artificial infection experiments by methods which will be explained below. For each variety listed one or more pots each containing numerous plants were inoculated. When infections were few or uncertain other pots of the same variety were tried. Uninoculated plants served as checks. Because of rapidity of growth as well as ease of handling, seedlings 6–12 inches high were largely used. (Numerous inoculation experiments show that maturer plants may be also infected.)

Wheat: Alabama Blue Stem, Coker's Blue Stem, Black Hulled, Fulcaster, Fultz, Georgia Red, Gladden, Golden Chaff, Gypsy, Harvester King, Hastings, Jones' Climax, Leap's Prolific, Lebanon, Marvelous, Medium Mediterranean, Michigan Wonder, Poole, Portage, Purple Straw, Red May, Red Wonder, Stover's Miracle, Turkey, Nebraska No. 6, and Turkish Amber.

No infections were obtained on Bartt, Beechwood Hybrid, Longberry, and Red Rock, but it should be noted that experiments would have to be repeated with these varieties before any deductions were made, since the temperature prevailing in the greenhouse at the time inoculations were conducted was considerably below the optimum for infection.

Oats: Appler, Ferguson, Virginia Turf, Wilson, Winter Gray, and Winter Turf.

Rye: Abruzzi, Rosen, Station, Texas Winter, and Winter Minnesota. Infections on Ivanhoff No. 34 not obtained.

Barley: Mancheuri, Oderbrucker, and Wisconsin Pedigree No. 6.

Corn: Arlington Prolific, Biggs' Seven Ear, Calhoun Red Cob, Chisholm, Coker's Ellis, Coker's Marlboro, Coker's Prolific, Coker's Williamson, Eureka, Experiment Station Yellow, Hickory King, Jarvis' Improved, Laguna Mexican June, McFarland, Mosby's Prolific, Sentell's White Dent, Singleton's Strawberry, Stewart's Yellow Dent, Surecropper, Thibault's Mexican June, Weekby's Improved, Whattey's Prolific, and White Wonder.

Varieties of corn upon which no infections were obtained are Brazos White Corn, Coker's Garric, Ewing's Mosby, Paymaster, Silvermine, and Southern Beauty. Time has not permitted any

[1]The writer wishes to express his indebtedness to the Agricultural Experiment Station of the University of Arkansas and in particular to Professor W. H. Sachs for supplying most of the seed of the varieties listed. The varieties are for a large part those that are adaptable to Arkansas conditions.

adequate study which would definitely show whether or not these are resistant.

Sorghum: Black Amber, Darco Non-saccharine, Honey, Red Amber, and Sugar Drip.

The following sorghum varieties yielded no infections: Broom Corn, Silvertop, Shrock Kafir, Sumac, and Sunrise Kafir. Here also conditions for infection were not the best.

SUMMARY OF THE WORK ON VARIETAL SUSCEPTIBILITY

The following points may be emphasized: first, that a comparatively large number of varieties of different cereals are susceptible; second, that the degree of susceptibility has not been clearly worked out; third, that certain cereals, like oats, barley, and rye, seem to show greater susceptibility, and conversely, that others, like corn and sorghum, are not as susceptible. The infection experiments here reported were done entirely in the greenhouse, and at times factors influencing infection, such as temperature, were difficult to control, so that conditions for infection were not uniform. The degree of susceptibility was measured by the number and size of the spots produced.

Isolation and Inoculation Experiments

The organism was not difficult to isolate, and when once its identity was established and the peculiar behavior of producing a colorless halo surrounded by a white precipitate on certain culture media was recognized, it was easy to detect it on a poured plate, even in the presence of various other *Schizomycetes*. During the 4 years in which this disease has been studied, the pathogen has been isolated many times from natural infections and from artificial infections on various hosts, using the ordinary method of surface sterilization with 1-1000 mercuric chloride solutions for about 2 minutes, washing in several changes of sterile water, macerating aseptically either in sterile water or in beef bouillon, diluting variously by successive transfers into sterile water blanks, and finally by making poured plates containing small amounts of the dilution culture in nutrient agar. When proper precautions are taken, such as thorough cleansing of hands, of clothing, and of the chamber in which the work is conducted it is common to get a series of plates in which the pathogen only is present.

Since the organism is very sensitive to alcohol, as will be shown later, attempts at surface sterilization with alcohol-mercuric chloride solution are apt to yield no colonies, although the writer has been able to obtain isolations in this way by a very rapid transfer of the diseased material from the alcoholic solution to the straight mercuric chloride solution.

In inoculation experiments the organism was at first smeared on the leaves by means of a sterilized platinum loop and in all

later work was applied as a spray. The first method has the advantage of enabling one to locate definitely the point of inoculation in relation to the point of infection, but it is rather tedious when any number of inoculations are to be attempted. Either agar or broth cultures (the age of the cultures, within limits, not being very important for this organism) were diluted with sterile water before the spray was applied. (In smear inoculations the plants were first sprayed with sterile water.) The spray was obtained by using atomizers or blowers commonly used by artists in "fixing" drawings. These work very rapidly and effectively, producing a fine mist with little effort.[1] The organism having been applied, the plants were then covered with bell jars to prevent drying out. The bell jars were ensconced with paper in order to cut down direct sunlight. The jars were left over the plants for 1 or 2 days, depending on how high the temperature happened to be. It may be worth recording that the difficulty experienced in obtaining infections in the experimental greenhouses of the Missouri Botanical Garden, although no such difficulty had been experienced at Fayetteville, Ark., was overcome by proper aëration. The greenhouse benches at the Garden are of concrete. When inoculated pots of plants were placed on soil which filled these benches, and the bell jars which covered the plants made close contact with the soil, infections were rare, no matter what the temperature and humidity happened to be. But by raising the jars from the soil on small blocks of wood the plants were still kept moist and at the same time had access to air. In this way infections were readily obtainable, other conditions being proper.

RELATION OF TEMPERATURE TO INFECTION

It has already been indicated that temperature plays an important role in infection. The reproduction of thermographs of artificial inoculations on Winter Turf oats carried out as previously described, illustrates this point (fig. 1).

The pot of plants subjected to the inoculation temperatures shown in the lower graph developed no infections, while that of the upper developed quite a few. It will be noted that where no infections were obtained the temperature over a large part of the 48-hour period was below 70° F. (21° C.), and particularly during the first 24-hour period the temperature was below 70° F. for 18 hours. The atmosphere was saturated and the leaves were covered with water films in both cases. It was found difficult to keep the incubator at an even temperature, so that no data are available which will show the relationship of infection to a definite temperature. Nevertheless, the graphs give some indication on this point and when one considers the difference

[1]Dr. J. A. Elliott first suggested these blowers to me. They are cheap, easily sterilized, take up little space and do not plug readily.

between night and day temperatures often encountered in the field the data shown are perhaps more indicative of infection to be expected under natural conditions than any experiment would show in which the temperature is kept at one level during the entire incubation period. Roughly then, it may be said that tem-

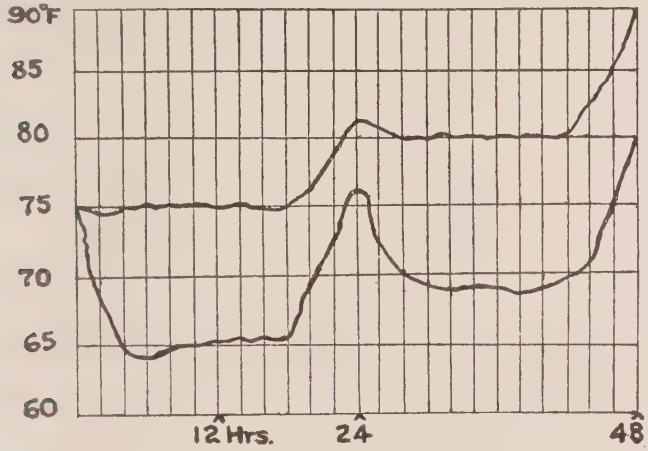

Fig. 1. Thermographs of artificial inoculations on Winter Turf Oats.

peratures during the infection and incubation periods, below 70° F. prevent infection, while temperatures above 75° F. are conducive to infection. It may be added that numerous artificial infection experiments show that heaviest infections occur at temperatures between 85 and 95° F. (29 and 35° C.). This is to be expected since growth in artificial culture media is best at these temperatures, as will be shown later.

THE ORGANISM

Morphology.—The organism is an actively motile, medium-sized rod. Whether obtained directly from the host tissue or whether grown on artificial media of various kinds no marked differences in morphology have been observed. It is rather slender (pl. 5, fig. 2) with rounded ends, and measures 0.5–0.8 × 1.5–2.0 μ, 0.6 × 1.8 μ being very common measurements. Very rarely is it observed other than singly or in pairs, although occasionally a short chain of 4 or 6 organisms is to be seen. Its motion, which is very rapid in warm water, is due to a single, polar flagellum which varies somewhat in length but is commonly 3 to 5 times the length of the organism. Van Ermengem's stain brings out the flagella, but unfortunately it does not stain the body of the organism properly (pl. 5, fig. 3). Pitfield's stain as well as Shunk's (H. '20) modification of Loeffler's stain, has also

shown but one polar flagellum. The latter stain, in particular, after the mordant has been seasoned for several weeks, forms an excellent stain for this organism, the disadvantage being that the blue color imparted by the methylene blue is difficult to photograph by ordinary methods.

The organism possesses capsules, as definitely shown by using Huntoon's (II, '17) method, "nutrose" being used as the diluent and Ziehl's carbol fuchsin added in addition to the stain recommended by Huntoon. This method, in contrast to Ribbert's or Welch's, is much more satisfactory in bringing out the capsule on this organism. Young and old cultures of the organism on nutrient agar slants and in bouillon were used, and capsules were observed in every case. No zoogloea or pseudozoogloea were formed, although in old cultures of beef-bouillon and particularly in bouillon containing various sugars, a dense precipitate settled, which on agitation rose in whorls and was broken up with difficulty.

The organism has not been found to produce spores. Cultures at various ages and on various media were examined. Anjesky's spore-staining method, recommended by Giltner ('Laboratory Manual,' 1921 edition) was used. It is not acid fast. A bouillon culture 3 days old stained with Ziehl's carbol-fuchsin and then treated with 20 per cent sulphuric acid for a moment, showed no color. When counterstained with aqueous methylene blue the organism appeared deep blue with no trace of red. It was Gram negative. A 24-hour culture treated with the Gram stain was colorless and became brown when counterstained with Bismarck brown. A check test with the same Gram stain on *Staphylococcus albus* showed this organism definitely stained. Ordinary stains, like gentian violet, carbol fuchsin, methylene blue, and Bismarck brown were taken up readily. No irregular or involution forms have been seen.

CULTURAL CHARACTERS IN RELATION TO ACIDITY AND ALKALINITY

Methods.—In making up culture media the recent recommendations of the committee (Conn, II, '20) representing the American Bacteriological Society, as far as they go, were followed, with modifications as will be noted, and in addition other methods and media were used which seemed worthy of trial. The aim has been to utilize the current methods of plant pathologists and at the same time take cognizance of technique employed by bacteriologists in general and by the medical men in particular.

The necessity of utilizing methods which are not commonly used by plant pathologists became evident early in the work, and having in mind the busy life of the ordinary pathologist, it will be necessary to indicate the reason for the adoption of any new scheme.

Using nutrient agar testing +10 or +15 on Fuller's scale (10 or 15 parts per 1000) the writer found that from diseased foxtail material he would at times obtain bacterial colonies which produced a very conspicuous reaction, a colorless zone followed by a marked whitish area (pl. 6, fig. 1), and then perhaps at the same time, on other plates, or at other times, using the same medium, colonies were obtained without any such features. Morphologically, the colonies as well as the individual bacteria appeared exactly alike, and inoculation experiments showed that both were pathogens producing the same kind of spot. Two explanations for this variation seemed plausible, first, that there existed 2 strains of the same species, one producing a precipitate and the other not, and second, that the conditions in the nutrient media were different. It was soon found that a colony that produced a white precipitate may, on transferring to another plate or tube, show no such character. It appeared therefore that there must be some slight difference in culture media even though it was made up at the same time and received the same treatment throughout.

In an effort to solve this, and mindful of the extensive data that were accumulating on hydrogen-ion concentration in relation to various phases of bacterial activity, the writer instead of titrating by the Fuller scheme, using phenolphthalein as the indicator, used the indicators recommended by Clark (II, '20) in which the color change with reference to acidity or alkalinity had been definitely worked out in terms of hydrogen-ion concentration. The color obtained by adding a few drops of indicator, like brom thymol blue, to a tube of melted, nutrient agar was then compared with the color chart given by Clark (following p. 40), and the color in the series from brom thymol blue matching best with the tube was taken as the hydrogen-ion concentration in terms of P_H. This of course is a rough way of measuring true acidity or alkalinity but its superiority over Fuller's method soon became evident. Nutrient agar which gave a yellow or yellowish green color with brom thymol blue (those covering the reaction range of P_H 6.0—P_H 6.8 and therefore acid) when used as a substrate always showed colonies producing a colorless region followed by a dense precipitate, while agar which gave a blue color with brom thymol blue (those having a reaction of P_H 7 or over, hence neutral or alkaline) when used as the substrate showed colonies which never produced any precipitate.

The presence or absence of a colorless zone plus precipitate could then be controlled at will, depending on whether the medium was rendered slightly acid or slightly alkaline as measured by the hydrogen-ion concentration. Here then is a method of measuring acidity and alkalinity which is just as simple as Fuller's and much more accurate. By using this method the writer was able to obtain a character which immediately distinguished

this bacterium from other similar-appearing organisms; in other words, using a medium of P_H 6.6, no matter what other white colonies might develop on a given plate, this organism could readily be recognized. (This power of producing a colorless zone and a white precipitate was an excellent character as a quick test for differential purposes).[1] The reason why Fuller's scale did not bring out this feature is apparent. A medium which tests +10 on Fuller's scale, and thus supposedly acid, is often, as will be shown later, really neutral or slightly alkaline. Besides this, it has been found that a medium which is slightly acid when it is prepared will change its reaction upon sterilization or upon standing. In particular the writer has found that nutrient agar kept in glass tubes of various "makes" will in time become alkaline.[2] This is especially true of much of the soft glassware obtained during the war, so that media that tested P_H 6 when made would, after two weeks, test P_H 7, and moreover, the same degree of change did not occur in different batches of tubes. New tubes in particular, even after hardening in several dippings of sulphuric acid-potassium dichromate cleanser and after several sterilizations in the autoclave, persisted in giving off considerable alkali. Hence in any series of 10 tubes, some made by one concern and some by another, or some old and others new, it was possible to obtain various acidities and alkalinities using the same medium. This in all probability is the reason for obtaining a series of poured plates with the same organism, some of the plates showing colonies with characteristic precipitate and others lacking in this feature. For the effect of autoclaving on the reaction of the medium see articles by Clark (II, '15, p. 130), Anthony and Ekroth (II, '16), Norton (II, '19) and Davis (II, '20). Besides glassware, other factors may change the reaction of a medium. Grace and Highberger (II, '20) noted changes in acidity, particularly in uninoculated glucose broth, and offered no explanation for the change. Foster and Randall (II, '21) found an increase in acidity of broth which they concluded was due not to a taking up of carbon dioxide, but to an opening up of the COHN groups as a result of the hydrolysis of the protein constituents.

Here it may be pointed out that not only has titration as measured by the hydrogen-ion concentration given more accur-

[1]Miss Bryan (II, '21) has recently described a bacterial bud rot of cannas in which she shows the pathogen producing a colorless zone surounded by a white precipitate on whey agar. Because of this similarity with the foxtail organism it was thought desirable to compare *Bacterium Cannae* with the foxtail pathogen. Miss Bryan very kindly furnished the writer with cultures of her organism. Comparison between the two show numerous differences, morphological and physiological, although as far as producing a precipitate is concerned they behave alike. The interesting thing about *B. Cannae* is that it produces a colorless area surrounded by a white precipitate not only on whey agar but also on ordinary nutrient agar possessing a slight acidity, as P_H 6.6. Furthermore, the production of this character can be controlled in the same manner as in the foxtail organism.

[2]Esty and Cathcart (II, '21) have recently given some interesting data on the effect of different glassware on various solutions.

ate results but the use of this method has stimulated careful analyses of physiological processes as related to active acidity or alkalinity with the discovery of numerous interesting and often valuable relationships, particularly with reference to human pathogens. A few instances will be cited. Clark and Lubs (II, '15) differentiated between organisms of the colon-aerogenes group by the use of indicators whose color changes were noted in terms of hydrogen-ion concentration. Ayers (II, '16) found that streptococci reached a more or less definite hydrogen-ion concentration in culture media, a fact which, he decided, helped in the classification of these bacteria. Later, in 1918, Ayers, Johnson, and Davis found that a non-pathogenic strain of *Streptococcus* reached a higher hydrogen-ion concentration than the pathogenic strains. They concluded that the difference in hydrogen-ion concentration may be used as one means of distinguishing non-pathogenic from pathogenic streptococci. Finally, Avery and Cullen (II, '19) found that they were able to distinguish human from bovine strains of *Streptococcus* because of the fact that human strains in nutrient broth develop a lower hydrogen-ion concentration. They were thus enabled to differentiate between those organisms that may be pathogenic to man and those that are not.

Fennell and Fisher (II, '19), working with cultures of pneumococcus and meningococcus, were often unable to obtain suitable growth although the media utilized had apparently the same composition, was made in the same way, and the titration by the Fuller method was always adjusted to the same point, +0.2 (0.2 per cent). Using the hydrogen-ion concentration in their titration they obtained profuse and consistent growth. They found that pneumococcus has a very narrow range for optimum reaction, P_H 7.8 to P_H 8.0, which they considered as equal to +3.0 to +3.5 (3 3.5 per cent), "a total acidity far beyond anything previously suggested for the growth of pneumococcus." Norton (II, '19) obtained similar results.

Not only has the hydrogen-ion concentration been used successfully for distinguishing between closely related organisms, but its use in the study of various phases in metabolism has yielded valuable results. Its relationship to the growth of pneumococcus and meningococcus has just been pointed out. In addition it may be noted that Cole and Lloyd (II, '17) showed that among other factors, a suitable hydrogen-ion concentration is important for the cultivation of gonococcus. There are now numerous records like these which show the relationship of growth and development of many different micro-organisms to hydrogen-ion concentration. Concerning its relationship to definite physiological reactions there are also numerous records; for example, Bronfenbrenner and Schlesinger (II, '19) pointed out that the amount of acid and gas produced by bacteria depend on vari-

ous factors, including the amount of carbohydrate, the amount of peptone, the amount of buffer, and on the hydrogen-ion concentration; Bigelow and Esty (II, '20) showed that a slight change in the hydrogen-ion concentration greatly affected the thermal death point, and Chambers (II, '20) gave accurate counts of the number of bacteria developed at different levels of hydrogen-ion concentration. Studies on non-pathogenic bacteria and fungi, as well as on higher plants, have shown similar relationships to hydrogen-ion concentration. The work of Allen (II, '19) on *Azotobacter*, Gillespie and Hurst (II, '18) on *Actinomyces scabies*, Rose (II, '19) on *Nummularia discreta*, and Duggar (II, '20) on nutrient solutions for higher plants, to mention but a few, has shown the importance of hydrogen-ion concentration in the metabolism of numerous and diverse organisms.

Clark in 1915 summed up the matter as follows (p. 109): "The rate of enzyme action, the stability of colloidal structures upon which cellular life depends, the solubility of many physiologically important compounds, as well as the structure and composition of media and the color of the indicators used in the adjustment of their reactions are all dependent in greater or less degree upon hydrogen-ion concentration."

While it is absolutely essential that plant pathologists recognize the importance of the determination of hydrogen-ion concentration, yet it should be recognized that other factors are equally as important and when it is desired to know the buffer strength of a medium it even becomes necessary to use total acidity titration. That there is danger of attributing too much to hydrogen-ion has been pointed out in recent articles by Brown (II, '21[a]), Jones (II, '20), and Traube (II, '21).

In spite of the very considerable amount of data that has accumulated on this subject, many plant pathologists, as has already been stated, have paid little attention to it. With very few exceptions, Wolf and his associates in particular (II, '21), those who have dealt with bacterial plant pathogens have more or less completely ignored all the recent advances in this phase of bacteriology. Having presented data showing the necessity for determining the hydrogen-ion concentration of bacteriological media the writer will attempt to outline very briefly the principles involved and the methods used. This has already been done in a number of articles and books; the aim in presenting the matter here is to make available to plant pathologists the information at hand and to call attention to the more important references.

PRINCIPLES INVOLVED IN THE DETERMINATION OF ACID AND ALKALI

To define the terms acid and alkali in relation to the color reaction of some organic indicator is not satisfactory. Thus, to say that a solution is acid because it shows no color in the pres-

ence of phenolphthalein does not give much information, since by using another indicator, such as Congo red, the same solution may be said to be alkaline (see Washburn, II, '10). The satisfactory basis for a definition of acidity and alkalinity is furnished by the ionic theory of Arrhenius (II, '13)[1]. According to this theory when a strong electrolyte dissolves in water in a dilute solution it is highly dissociated into two ionic groups. Hydrochloric acid, for example, breaks up into positive + H ions and negative — Cl ions, and sodium hydroxide breaks up into + Na ions and —OH ions. When a solution of hydrochloric acid is mixed with a solution of sodium hydroxide the hydrogen ions combine with hydroxyl ions to form water and in this union neutralization takes place. Pure water itself dissociates into + H and — OH ions, and the dissociation yields equal amounts of + H and — OH ions. A neutral solution may therefore be defined as "one in which the concentration of hydroxyl ions is equal to the concentration of the hydrogen ions. From this it follows that an acid solution is one in which the concentration of hydrogen ions exceeds that of OH ions, while an alkaline solution is one in which the concentration of hydroxyl ions exceeds that of hydrogen ions." Strong acids and alkalis like hydrochloric acid and sodium hydroxide are almost completely dissociated at certain concentrations, while weak acids like acetic acid and weak alkalis like ammonium hydroxide are only slightly dissociated.

The extent to which substances are dissociated into their ions may be determined by the hydrogen electrode and by other methods and this determination, in which the hydrogen- and hydroxyl-ion concentration is definitely determined, becomes a measure of the acidity or alkalinity of the solution.

Normal solutions of hydrochloric acid and of acetic acid will neutralize the same amount of sodium hydroxide, but since hydrochloric acid is a strong acid it is almost completely dissociated, while the weak acetic acid is but weakly dissociated. The result is that normal hydrochloric acid, according to Michaelis (II, '14, p. 23), contains about 0.8 gram of hydrogen per liter, while normal acetic acid contains only 0.0043 gram of hydrogen per liter. The difference between the 2 acids may be noted in table I, taken from Michaelis (II, '14, p. 23).

As has already been indicated, water itself is an electrolyte and dissociates into + H ions and — OH ions. Sörensen determined that pure water at 22° C. contains a concentration of 1/10,000,000 of either ion, that is, that it contains 0.0000001 gram of H ions and the same amount of OH ions per liter. In other words pure water is 0.0000001 normal acid and at the same time 0.0000001 normal alkali. Such fractions as 1/10,000,000 (0.000-

[1]This author, in a comparatively simple account, has summarized in English, the theories of solutions. The book is easy to read and is not burdened with mathematical formulae.

0001) being unwieldy, the logarithmic equivalents are used; thus 1/10,000,000 may be written 10^{-7}, and the hydrogen-ion concentration of theoretically pure water may then be written 10^{-7}, or expressed in the form of a negative logarithm as Sörensen does, $P_H 7$, which denotes the pressure of hydrogen ions (written variously pH, P_H, p_H^+, the last being Sörensen's original method [II, '09, p. 4]) or, in other words, the hydrogen-ion concentration.

TABLE I

H-ION CONCENTRATION OF STANDARD SOLUTIONS OF ACIDS

HCl at 18° C.			Acetic Acid at 18° C.		
Concentration	H-ion concentration	P_H	Concentration	H-ion concentration	P_H
Normal (1.0)	0.8	0.10	Normal (1.0)	4.3×10^{-3}	2.366
0.1 N	0.084	1.071	0.1 N	1.36×10^{-3}	2.866
0.01 N	0.0095	2.022	0.01 N	4.3×10^{-4}	3.366
0.001 N	9.7×10^{-4}	3.013	0.001 N	1.36×10^{-4}	3.866
0.0001N	9.8×10^{-5}	4.009			

At a given temperature the product of the concentration of H and OH ions is constant and this value is called the "dissociation constant." For pure water at 22° C. this is equal to 10^{-14}, since, as has already been stated, the concentration of H ions and of OH ions is each equal to 10^{-7}. As the dissociation constant at a given temperature is always the same, the concentration of either +H or —OH can be expressed in terms of the other. Thus 1/100 N hydrochloric acid which has an hydrogen-ion concentration of 10^{-2} and an hydroxyl-ion concentration of 10^{-12} is said to have a value of P_H 2. Similarly, a 1/100 N sodium hydroxide solution which has an hydroxyl-ion concentration of 10^{-2} and an hydrogen-ion concentration of 10^{-12} has a value of P_H 12. All acids having a greater hydrogen-ion concentration than pure water have a value less than P_H 7 (the smaller the logarithmic exponent of a number less than 1 the greater the number); conversely all alkaline solutions having a lower hydrogen-ion concentration than pure water have a value greater than P_H 7. Table II adopted with modifications from Medalia (II, '20, p. 433) may help in an understanding of the matter.

Good accounts of the principles involved in hydrogen-ion concentration may be had in the following: Sörensen (II, '09), Washburn (II, '10), Michaelis (II, '14), Clark (II, '15, '20) and Committee on Descriptive Chart for 1918 (II, Conn, '19).

TABLE II

RELATION OF P_H VALUES TO STRENGTH OF SOLUTION

Strength of solution	Grams of hydrogen per liter	Expressed logarithmically	P_H value	
Normal HCl	1.0	10^{-0}	0.0	Acid
1/10 N HCl	0.1	10^{-1}	1.0	Acid
1/100 N HCl	0.01	10^{-2}	2.0	Acid
1/1000 N HCl	0.001	10^{-3}	3.0	Acid
1/10000 N HCl	0.0001	10^{-4}	4.0	Acid
1/100000 N HCl	0.00001	10^{-5}	5.0	Acid
1/1000000 N HCl	0.000001	10^{-6}	6.0	Acid
Pure water	0.0000001	10^{-7}	7.0	Neutrality
1/1000000 N NaOH	0.00000001	10^{-8}	8.0	Alkaline
1/100000 N NaOH	0.000000001	10^{-9}	9.0	Alkaline
1/10000 N NaOH	0.0000000001	10^{-10}	10.0	Alkaline
1/1000 N NaOH	0.00000000001	10^{-11}	11.0	Alkaline
1/100 N NaOH	0.000000000001	10^{-12}	12.0	Alkaline
1/10 N NaOH	0.0000000000001	10^{-13}	13.0	Alkaline
Normal NaOH	0.00000000000001	10^{-14}	14.0	Alkaline

METHODS OF MEASURING HYDROGEN-ION CONCENTRATION

The hydrogen-ion concentration, that is, the true acidity of a solution, may be measured in various ways. The two common methods used are, first, by measuring the electromotive force of a solution, and second, by the use of certain indicators whose color changes in relation to various levels of hydrogen-ion concentration have been definitely determined by the first method. The first or electrometric method, with good equipment, is more accurate, while the second or colorimetric method, while not as accurate, is much more available, since the cost is much less, and its operation very much more simple. (The writer having seen both in operation is satisfied that for ordinary work, and even for special research, the first is not essential for a bacteriologist.) For references to methods and apparatus necessary for electrometric measurements see Clark (II, '20) and Leeds and Northrup Company, Catalogue No. 75 (II, '20).

Indicators developed by Clark and Lubs ('17) for colorimetric work are especially well fitted for bacteriological media since they are as a whole brilliantly colored, their color changes at different levels are marked, and they are readily procurable at reasonable rates. Their color changes with reference to hydrogen-ion concentration have been carefully ascertained by Clark and Lubs and the values given by these authors have been checked up by numerous investigators. Explanation of the colorimetric method may be had in Friedenthal (II, '04), Salm (II, '04),

Sörensen (II, '09), Clark (II, '15, '20) and Committee on Descriptive Chart for 1918 (Conn, II, '19).

STANDARDS FOR COMPARISON

Having added a few drops of an indicator, like brom thymol blue, to a tube of nutrient broth, how is the P_H value to be determined? The writer has already indicated one method, namely, comparing the tube with the color chart given by Clark (II, '20). This method for general laboratory work was found very satisfactory; the error was small, particularly if the solution was not highly colored; it was comparatively easy to manipulate and could be used readily in the class room; and best of all, it was very simple and comparatively inexpensive. The errors involved in this method are: first, it is difficult to get 2 charts which exactly agree in the shades of color for any one indicator, second, any color in the solution to be tested interferes with the color of the indicator. Nevertheless, in the absence of definitely known standards, this method is to be preferred, in the opinion of the writer, to the method advocated by the Committee on the Descriptive Chart (Conn, II, '20). This committee recognized the value of the Clark and Lubs indicators but instead of using anything for comparison, they simply recommended the following: "Bring the media to such an acidity as to turn this indicator (brom thymol blue) a distinct grass-green (neither yellow green nor blue green)."

It is evident from what has been said that it is desirable to have some standards whose hydrogen-ion concentrations have been definitely ascertained. Standards such as those recommended by Clark and Lubs contain certain salts, such as borates, phosphates, phthalates, which in the presence of certain indicators present definite colors. (The hydrogen-ion concentration of these standards having been previously determined by Clark and Lubs and by others using the electrometric method, it thus becomes possible in performing a titration to obtain a series of solutions of known hydrogen-ion concentrations which possess definite colors at certain levels of hydrogen-ion concentration.) The standards largely developed by Sörensen, recommended by Clark and Lubs, and in common use are difficult and laborious to make, but for careful work, are apparently indispensable. Using these standards and a comparator block, it is a simple affair to compare a tube of media containing a certain indicator with another tube containing the standard solution plus the same indicator. This method, slightly modified by placing a tube of clear water before the solution to be tested and a tube of the unknown solution without indicator before the standard, as recommended by various investigators, has been extensively used by the writer in this investigation. The procedure is fully described by Clark (II, '20).

Various devices designed to simplify the procedure in making standards have appeared; most of these consist in reducing the number of standard solutions, which in some instances, also reduce the range of P_H values (see McIlvaine, II, '21). One of the simplest of these, judging from the description, is the one advocated by Acree and his associates (II, '21). The writer has not been able to use this. Other attempts to reduce the labor involved in making standards consist in varying the number of drops of indicator in a series of tubes containing simply a few cubic centimeters of acid and alkali. This was first recommended by Barnett and Chapman (II, '18) and later amplified by Medalia (II, '20) and by Gillespie (II, '20). Bunker and Schuber (II, '22) claim good results by this method, and it is the one recommended by the American Public Health Association in the "Standard Methods for the Bacteriological Examination of Milk," 1921.[1]

BUFFERS

Standard solutions used for comparison with unknowns are often called "buffers". What is a "buffer"? Any solution which resists change in hydrogen-ion concentration upon the addition of acid or alkali is called a "buffer". It is of course desirable that standard solutions once made up should retain their calculated hydrogen-ion concentration as long as possible, and certain salts in particular are chosen for standards because of their marked resistance to change in hydrogen-ion concentration, even after standing for several months. Not only do a large number of organic and inorganic salts act as buffers but many other substances, such as peptone, beef extract, blood, etc., act in a similar manner. A good account of buffer action appears in Clark's work (II, '15, p. 116).

COMPARISON BETWEEN FULLER'S SCALE AND HYDROGEN-ION CONCENTRATION

Having briefly and perhaps inadequately described what is meant by hydrogen-ion concentration and how to measure it, the next question worthy of attention is, how does the older titration method, such as Fuller's (II, '95), compare with the determination of hydrogen-ion concentration. There are two main reasons why the two methods yield different results; first, the difference in degree of dissociation of different electrolytes; and, second, the buffer action of various substances. As previously described, when equal amounts of normal solutions of hydrochloric and acetic acids are titrated the same amount of alkali is utilized, although the two acids have entirely different P_H values, since

[1]Ready-prepared standard solutions are advertised by La Motte Chemical Products Company, 13 W. Saratoga St., Baltimore, and by Graham Chemical Company, 100 Rockingham Street, Rochester, N. Y. The latter concern sells the product developed by Acree and his associates ('21).

one dissociates very strongly and the other but weakly; that is, titrating with sodium hydroxide, in the case of weak acids, gives no indication as to the actual acidity or the hydrogen-ion concentration. When a nutrient medium is neutralized with strong alkali the figure obtained gives no indication of the actual acidity present in the medium but gives an expression of the total acidity, including the "active" acidity and the "reserve" acidity, and this "reserve" acidity remains an unknown quantity made up of undissociated acid molecules as well as the acid held in union by the buffers present in all ordinary nutrient media. Titrating with sodium hydroxide then gives a measure of the total acidity, while the hydrogen-ion concentration measures the true or "active" acidity.

Why is it insufficient to titrate for total acidity? The following illustration taken from Sörensen (II, '09) answers this question. He and other investigators found that certain enzymes, such as invertase, catalase, pepsin, and others, show optimum activity in the presence of a certain amount of acid, but the quantity of acid necessary for this activity could not be definitely ascertained by the ordinary titration method. The reason for this is clear. A solution of an enzyme, such as invertase, contains substances which are capable of combining with acids, so that the optimum acidity depends, for one thing, on the substances, buffers, going with the enzyme. However, without measuring the hydrogen-ion concentration it is not possible to get a measure of the exact amount of these substances so that it becomes impossible to indicate the optimum degree of acidity which would be the same under all experimental conditions. On the other hand, obtaining a measure of the acidity by means of the hydrogen-ion concentration, it is possible to show that this is constant and is as definite as the nature and quantity of the enzyme. Thus, while the total acidity necessary to give optimum enzyme action is very different for 3 enzymatic solutions, the optimum concentration of hydrogen ions, on the other hand, is the same for all 3.

Using a similar illustration for bacteriological media, it may be said that while the optimum acidity for the growth of a particular organism may be +15 on a medium containing Witte's peptone, it will be different on a medium containing some other peptone. When an organism is found to respond best to a reaction of P_H 7, it will always respond similarly at that reaction no matter what the peptone may be, other things remaining the same. Occasionally it becomes important to know the buffer value of the media and in such cases a determination of the total acidity by ordinary titration is utilized in conjunction with a determination of the hydrogen-ion concentration. Illuminating articles on the buffer values of different peptones have been written by Bronfen-

brenner, DeBord, and Orr (II, '21) and by Brown (II, '21).

Titration (Fuller's scale)	P_H
+ 7.5	7.5
+ 8.0	7.4
+10.0	7.5
+11.5	7.1
+12.5	7.4
+14.0	6.8
+14.5	6.8
+15.0	6.9

Not only are different results to be expected because of the difference in principles in titrating for total acidity as compared to a determination of hydrogen ions, but total acidity measurements, such as the procedure involved in calculating Fuller's scale, are rather inaccurate for other reasons. As Clark (II, '15) has stated, the use of phenolphthalein as indicator, titrating when the medium is hot, and the difference in end point used by different investigators due to lack of proper color standards, all tend to make the ordinary titration method variable and inexact.

Since Fuller's scale has had such common usage it seems desirable to present actual comparisons of this scale with the determination of hydrogen ions. Various investigators have given comparative figures, the table given by Norton ('19) and reproduced above, is one illustration of such comparison in bacteriological culture media.

The titrations were made by the ordinary methods using phenolphthalein as indicator. Attention should be called to the fact that figures under titration represent parts per thousand as recommended originally by Fuller instead of parts per hundred as is used by Norton.[1] It will be noted that while the total acidity titration (Fuller's scale) shows the media to be markedly acid, the acidity in terms of hydrogen-ion concentration is very nearly neutral or even alkaline. Figures obtained by me show the same thing. A few are here listed:

	P_H	Fuller's scale
Nutrient beef broth acidulated with 0.1% citric acid	4.8	+24.0
Nutrient beef broth acidulated with 0.05% hydrochloric acid	5.5	+12.0
Nutrient beef broth neutralized with sodium hydroxide	7.0	+10.0
Nutrient beef broth rendered alkaline with sodium hydroxide	7.2	+ 6.0

Here also a medium which is neutral or even alkaline with reference to hydrogen-ion concentration appears acid in Fuller's scale. This of course is not surprising since, as is now well

[1] A discussion of the difference in interpretation of Fuller's scale is given by the writer in another publication (Rosen, II, '22).

known, the color change of phenolphthalein instead of being at the neutral point, is actually on the alkaline side, between P_H 8 and P_H 9.6, so that media below P_H 8 would appear acid if this indicator is used. It is necessary to emphasize the fact that these comparisons are merely indications of what differences may be expected. Any changes, such as the amount or kind of peptone or of beef extract, will change the P_H value even in the presence of the same amount of acid. In these comparisons the medium used consisted of 3 grams of Liebig's beef extract, 10 grams of "Bacto" peptone, and 1000 cc. of distilled water.

GROWTH OF THE FOXTAIL ORGANISM ON VARIOUS MEDIA

Two different kinds of agar were used, the Difco powdered agar and a shredded agar of unknown make. No difference in growth was noted but the first is to be preferred since it is more readily soluble. Difco peptone was used and two different kinds of meat extract, dehydrated Bacto beef, and Liebig's meat extract. The dehydrated beef gave better growth but required much longer time to make and resulted in a turbid, dirty product, making hydrogen-ion determinations by indicators very difficult.

Agar Plates.—On peptone-beef agar poured plates, P_H 6.6, kept at 20° to 25° C., colonies appeared within 48 hours. They were white, round, smooth, and in 4 days well-separated surface colonies measured as much as 6 mm. in diameter. At first they showed a bluish tinge but later became opaque white with a somewhat faintly colored creamy yellowish center. The margin was entire, with a conspicuous colorless zone 2 to 4 mm. wide surrounded by a marked whitish discoloration of the medium which was most conspicuous at the border of the colorless zone and gradually faded out into the color of the surrounding medium. The whitish discoloration might be recognized at a distance of 0.5 cm. from the colorless zone at the end of the fourth day although it was faint at this distance. The colony was somewhat sticky to the touch of a needle but was not viscid; it was glistening, somewhat raised or convex, and internally appeared amorphous, that is, without distinctive markings. As the medium dried out the colonies became indistinct and gradually disappeared from view. Buried colonies made but little growth. On P_H 7.2 peptone-beef agar growth was the same but there was no colorless zone or precipitate.

Agar stroke.—At 25 to 30° C. there was a moderate amount of growth in 48 hours, often reaching a width of around 4 mm. at the base of the slant and tapering upwards. The growth was filiform, raised, glistening, smooth, and opaque white to creamy white, odor was absent or not marked, and the consistency was that of melted butter. Growth was very marked at the base of the slant and in the water of condensation and of syneresis. As

the smears became old there was a tendency to form a slight, thin growth at the margin which upon close examination appeared in the form of irregular, fine, thread-like projections. Below P_H 7, a colorless region surrounded the smear and this region in turn was bordered by a dense white precipitate. This precipitate became very marked by the third or fourth day. Above P_H 7 there was no colorless zone or precipitate.

Agar stab.—There was good growth at the surface and slight growth in the upper part of the stab. There was no growth in the lower part of the stab.

Gelatin plates.—On poured plates, kept at about 20° C., testing P_H 6, colonies appeared slowly. In 48 hours they were barely visible, being in the form of fine white points. By the fifth day well-separated surface colonies measured 1.5 to 2.5 mm. in diameter; they were round, the margin was entire, appearing coarsely granular when viewed with the low power of a microscope; the granules were not arranged in any definite pattern but were somewhat larger toward the middle of the colony. The color was glistening white with a bluish lustre towards the margin and a yellowish tinge in the middle. There was no liquefaction and no odor. In contrast to surface colonies, which possess even margins, colonies which were imbedded presented uneven margins, and were often deeply lobed. These colonies appeared very rough under the microscope, and the granules were much larger than those of surface colonies. Imbedded colonies were also much reduced in size. On P_H 7 gelatin plates the growth was the same as on P_H 6 gelatin. As the colonies advanced in age the granulation so evident up to the fifth day gradually disappeared. When plates were kept at 25 C., a temperature which caused a slight softening of gelatin testing P_H 6–7, the growth was noticeably better, well-scattered colonies measuring 5 to 6 mm. on the seventh day; as the gelatin softened the surface colonies sank into the medium and took on concentric striae, a character not evident on firm media. Even after 2 months there was no liquefaction. In contrast to gelatin testing P_H 6.0 and P_H 7.0 there was no growth on gelatin testing P_H 5.5 (P_H 8.2 gelatin refused to gel when it had been rendered alkaline before sterilization). On gelatin growth as a whole was not as good as on agar but it should be noted that this might be due to the comparatively low temperature at which gelatin is kept, the organism being one which grows best at a comparatively high temperature. No colorless zone with white precipitate has been noted on gelatin.

Gelatin stabs.—Growth was visible only in the upper part of the stab. There was no liquefaction.

Plain gelatin stabs.—As there is a possibility that the pep-

tone and beef extract of nutrient gelatin may interfere with the enzymes which liquefy gelatin, stabs were made in a medium containing no nutrients except the 1.5 per cent gelatin. On these the growth was but slight and in the upper part of the stab. Here also there was no liquefaction.

Nutrient broth.—Peptone-beef broth testing P_H 7 at 25° C. produced but a faint surface growth and a slight clouding of the medium in 24 hours. In 48 hours the surface growth was more definite and there was a marked clouding throughout the medium, there was no odor, and there was a flocculent sediment. By the end of the sixth day there was a marked surface growth as well as an irregular, granular growth in the upper part of the medium. On agitation these readily broke up into very finely divided flocculent particles. In old cultures there was a heavy sediment which when agitated rose in thread-like whorls and was broken up with difficulty. (One per cent of peptone was but slightly better than 0.5 per cent.)

Nutrient broth over chloroform.—As compared to growth in nutrient broth in the usual atmosphere, growth in an atmosphere of chloroform was much restricted, there was no surface growth and only faint clouding, but the sediment was the same in amount and kind.

Nutrient broth plus alcohol.—Absolute alcohol was added to nutrient broth in sufficient quantity to make solutions of 4, 5, 6, 7, 8, and 9 per cent. There was no growth in any tube above 4 per cent at the end of 10 days. While this gives no exact information on the actual percentage of alcohol which may inhibit growth, no provision having been made for the volatilization of the alcohol or to account for any combination with substances contained in nutrient broth, it nevertheless indicates the inhibiting action of this substance at relatively low concentrations and is quite comparable to the results obtained by others on non-spore-forming organisms.

Sodium chloride in nutrient broth.—One per cent sodium chloride had no marked influence on growth; with 2 per cent there was a reduction in growth and when 4 per cent was reached there was no growth. In 2 and 3 per cent broth there was a marked tendency to form long streamers which hung down from the surface and broke up slowly when shaken. This character is doubtless in the nature of a response to unfavorable, high, osmotic concentrations and indicates a tendency of the organism to remain united in chains and in clumps under such conditions.

Toleration of acids.—The acids used were acetic, citric, hydrochloric, malic, phosphoric, and tartaric. Various investigators have concluded that the germicidal effect of acids may be due to one or more of the following factors: the hydrogen ions, the

anions, and the undissociated molecules, the last 2 often being considered as important in the action of organic acids. The subject has received considerable attention and good summaries may be had in articles by Chambers (II, '20), Foster and Randall (II, '21), Schoenholz and Meyer (II, '21), and Wolf and Shunk (II, '21, pp. 14-20). It is of course clear that there is very little significance in simply adding 0.05 or 0.1 per cent of the acids employed without ascertaining what the active acidity of the media has become. When this acidity is measured it is then possible to compare fairly accurately the effect of one anion with another, at least at the same hydrogen-ion concentration. The writer has endeavored to follow out the old methods by measuring out 0.05 and 0.1 per cent of acid, adding this to nutrient broth, and then ascertaining by colorimetric comparisons what the hydrogen-ion concentrations are. Comparatively equal amounts of inoculum, 0.1 cc. of suspension from broth cultures 48 hours old, were used in the experiments made in Jena glass tubes, having ascertained that these tubes had no appreciable effect on the P_H value after sterilization. Twenty cc. of medium were used in each tube. The broth was rendered neutral, P_H 7, with sodium hydroxide before sterilization (tests after sterilizing exhibiting the same P_H). The acids were added aseptically after sterilization, the tubes being then incubated for 48 hours before inoculation in order to make certain that contaminations had not occurred.

TABLE III

EFFECT OF ACIDS ON GROWTH, MEDIUM NUTRIENT BROTH

Acid	Per cent	H-ion concentration of medium	Growth at end of 48 hours	Growth at end of 6 days	Growth at end of 9 days
Acetic	0.05	4.9	None	None	None
	0.1	4.8	None	None	None
Citric	0.05	5.0	Good	←	←
	0.1	4.8	None	None	None
Hydrochloric	0.05	5.5(?)	Very good	←	←
	0.1	4.5	None	None	None
Lactic	0.05	5.0	Good	←	←
	0.1	4.8	None	None	None
Malic	0.05	5.0	None	Fair	Good
	0.1	4.7	None	None	None
Phosphoric 85%	0.05	4.9	None	None	None
	0.1	4.3	None	None	None
Tartaric	0.05	4.9	None	Fair (1 out of 3)	←
	0.1	4.5	None	None	None

From table III there is a slight suggestion that acetate and phosphate ions are markedly injurious to growth, while malate and tartrate ions exercise a very retarding influence; in contrast to these the citrate, chloride, and lactate ions have no marked effect. It should be noted that there was no growth in any of the acids tried at the end of 24 hours although neutral broth kept at the same temperature, 25° C., showed marked clouding at the end of that period. This is to be expected since this organism in nutrient broth grows best around the neutral point, between P_H 6 and P_H 8. It is rather surprising to see the phosphate act as a poison and this perhaps needs to be repeated before accepting the result although the work was done carefully and carried out in triplicate. More will be said of this later. As far as the acetate is concerned the result obtained substantiates the findings of others (see, for example, Wolf and Shunk [II, '21, pp. 14-20]).

TABLE IV

CHANGE IN P_H AFTER 10 DAYS GROWTH IN ACID BROTHS

	Original P_H	Final P_H
Citric acid	4.9	7.4
Hydrochloric acid	5.5	7.4
Lactic acid	5.3	6.8
Malic acid	5.0	6.8

Table IV is of interest not only because it shows the production of alkali in this medium, a marked character of this organism, but also because it shows that in a given period of time the final hydrogen-ion concentration is not necessarily the same even though the initial concentration may have been quite similar. It is seen that there are apparently 2 groups, one developing an alkalinity of P_H 7.4, and the other shifting only to P_H 6.8. Why lactic acid should produce a higher final hydrogen-ion concentration than citric acid is a question; perhaps this is due to a difference in the dissociation of salts formed, but in the case of malic acid this is doubtless coupled with a lower amount of growth in the same time period (see table III).

It has already been noted that media will change on standing and that changes may be due to glassware or to some rearrangement in the chemical make-up of the medium. The changes as noted in table V are not very great; yet in some of these it is such that if the hydrogen-ion concentration were the only limiting factor the organism ought to grow in the changed medium.

TABLE V

CHANGE IN P_H OF ACID BROTHS AFTER STANDING 10 DAYS UNINOCULATED

Acids	Original P_H	Final P_H
Acetic	4.9	5.3
	4.8	4.8
Citric	5.0	5.3
	4.8	5.0
Hydrochloric	4.5	5.0
Lactic	4.8	5.0
Malic	4.7	5.0
Phosphoric	4.9	5.2
	4.3	4.8
Tartaric	4.9	5.3
	4.5	4.6

For example, citric acid broth changed from P_H 4.8 to P_H 5.0, an hydrogen-ion concentration not sufficient to prevent growth (see table III), yet when citric acid broth testing P_H 5.0, originally P_H 4.8, is inoculated there is no growth. The same is true for lactic, malic, and tartaric acids. Without more experimental data it is not possible to give an acceptable explanation of the change, which in this whole series is toward the alkaline side. There is of course some possibility that even with Jena glass there is a slight amount of alkali produced, particularly in strongly acid media; other possibilities such as the production of compounds in which a greater proportion of OH ions is set free may also be considered.

To test further the effect of the anion of various acids, 0.5 cc. of 0.405 N sodium hydroxide was added aseptically to 20 cc. of such acid media as had previously given no growth. (The alkali had first been sterilized so that there was no need of autoclaving after it had been added.) The results are shown in table VI.

Table VI, in comparison with table III, brings out very sharply the poisonous effect of the acetate anions when the hydrogen ions are not the limiting factors. However, it would appear, judging by the results of the phosphoric acid tests, that there may be limiting factors other than the cation and the anion. For one thing, it is hard to believe that the phosphate radicle, as such, has any poisonous action in the concentrations used (see account of growth in Fermi's solution). Of course, the undissociated molecule may play the role of poison but this also fails to give a convincing explanation of the action. The specificity of the organism with relation to phosphate may be a factor but this is

TABLE VI

ACID BROTHS WITH ALKALI ADDED

Acid	Per cent	P_H after addition of alkali	Growth at end of 10 days
Acetic	0.05	7.0	Good
	0.1	5.7	None
Citric	0.1	6.0	Good
Hydrochloric	0.1	7.4	Good
Lactic	0.1	7.0	Good
Malic	0.1	6.0	Good
	0.05	7.0	Good
Phosphoric	0.1	5.8	Good
Tartaric	0.1	6.2	Good

merely assumption; there may be some chemical rearrangement involved which has not been previously recognized, or the action may be a physical one involving such phenomena as surface tension. Traube (II, '21), for example, believes that surface tension should be considered as entering into the action of acids and bases.

In order to test the effect of autoclaving, acids were added to the medium before sterilizing. Approximately 15 pounds pressure for 20 minutes was used. The following acids were employed: citric, hydrochloric, lactic, malic, phosphoric, and tartaric. They were added to nutrient broth in amounts so as to make 0.1 and 0.2 per cent by volume. In every case the hydrogen-ion concentration, ascertained after sterilizing, was the same in each acid, and at both 0.1 and 0.2 per cent it was P_H 5.2. (The nutrient broth in this case contained dehydrated beef instead of Liebig's beef extract, which was used in the acid experiments previously reported.) No growth was obtained in any of the acid media, either at 0.1 or 0.2 per cent, while the same medium without acid showed excellent growth.

Comparison between cotton and glass wool as filtering agents.—In 1916 Miss Lloyd reported that certain substances necessary for growth of *Meningococcus* were removed when filtering was done through filter-paper in contrast to glass wool which, when used as a filter, did not absorb these substances. Since the filtering done by the writer, and as commonly practiced, is through absorbent cotton, it seemed desirable to test the effect of glass wool as compared with cotton on the growth of the foxtail organism. Using nutrient agar, adjusted to P_H 7, as the medium no difference in growth was detected, and in another series in which it was desired to obtain a comparison between distilled water and tap water in a medium of nutrient agar each kind of medium was

divided into 2 parts, 1 filtered through cotton and the other through glass wool. No difference in growth was evident either when tap water was contrasted with distilled water or when glass wool was used instead of cotton. In still another series, using nutrient agar as the medium, the 2 different filtering agents were used and part of the medium sterilized by the intermittent process in the Arnold sterilizer and the other part sterilized in the autoclave at 15 pounds pressure for 30 minutes. It was thought that if any "growth accessories" or "hormones" were involved a difference in sterilizing temperatures in conjunction with different filtering agents might show a difference in growth, but no difference was observed. It may perhaps be concluded that the foxtail organism does not respond to "growth accessories" although further work is necessary before this is accepted.

Loeffler's blood serum.—Growth restricted, whitish, glistening, filiform, slightly raised, and smooth; the medium was not liquefied.

Lima bean agar slants.—Growth in this medium was excellent; it was fully as good, if not superior, to any other medium tried, and bears out the suggestion made by others of making greater use of vegetable media in the study of bacterial pathogens. (The medium consists of 100 grams of dry lima beans, 15 grams of agar, and 1000 cc. of distilled water.) The beans were cooked until they readily fell apart and were strained through cheesecloth, the cloth being squeezed so that all the available liquid was recovered. (The medium as it finally appeared was rather turbid.) At 30° C. there was marked growth in 48 hours; it was raised, glistening, whitish, filiform, and there was no colorless zone or precipitate (P_H value not tested). As the culture became several days old a slight pinkish color developed in the smear, which was particularly noticeable at the base of the slant.

Corn meal agar slants.—Growth not as good as on lima-bean agar (ingredients in the same proportion as in lima-bean agar); it was filiform, quite the same whitish color as that possessed by the medium itself, and somewhat raised and glistening.

Whey agar slants.[1]—Growth at 30° C., medium testing P_H 5.4, was good; it was raised, filiform, ivory-yellow in color (see Ridgway, Color Standards, 1912), glistening, margin slightly undulating or entire, odor none, consistency slimy in the moist part at the base of the slant, and butyrous, somewhat sticky, in the remainder; there was a tendency to produce small, inconspicuous warts which appeared as whitish dots in a vitreous, ivory-yellow matrix. Surrounding the smear there was at first a colorless zone followed by a white precipitate, but by the fourth day the colorless area disappeared, due to an extension of growth at the mar-

[1]Made up according to the formula given by Miss Bryan (II, '21, p. 149) except that Bacto-gelatin was used instead of Nelson's photographic gelatin, No. 1.

gin overgrowing the area. The margin finally appeared markedly white in contrast to the interior, this whiteness being due to the white precipitate which had previously been formed when the smear was narrower. (By carefully removing the bacterial smear it was seen that the white portion coincided with the region where precipitate had been formed.) Growth on whey agar slants testing P_H 7.0 and P_H 7.2 was slightly better than on P_H 5.4 medium, but there was no colorless region and no precipitate. On P_H 9.0 whey agar there was no growth.

Uschinsky's solution.—Very slight growth in 3 days kept at 30° C., medium testing P_H 7.6; at the end of 10 days there was a marked ring with a faint surface growth and the fluid was rendered markedly viscid.

Fermi's solution.—Noticeable surface growth in 3 days which broke up into very fine particles on agitation and left a ring around the tube; there was but slight clouding. At the end of 7 days there was a very marked surface growth and a dense clouding in the upper two-thirds of the medium.

Cohn's solution.—No growth.

Potato cylinders.—Growth at 25-30° C. good, glistening, buckthorn-brown (Ridgway), sticky, slightly viscid. After growth of 1 month the cylinders were macerated without difficulty, 10 cc. of distilled water was added to each tube, and, in addition, 1 cc. of potassium iodide. A reddish purple color immediately developed which upon standing for several hours entirely disappeared, leaving a colorless mass; this was in contrast to uninoculated cylinders and in cylinders upon which *Bacillus Coli* was grown, in which the color remained deep blue.

Nutrient broth plus 1 per cent potato starch.—At the end of 11 days, 1 cc. of alcoholic solution of iodine was added and a reddish blue color developed which disappeared after standing over night. (*B. Coli* growth remained deep blue).

Nutrient agar plus 1 per cent potato starch.—Smears were made across the middle on plates containing nutrient agar plus starch. At the end of 10 days plates were flooded with a potassium iodide solution resulting in a clear zone about 0.5 cm. wide around the smears, while the remainder of the plates were deeply colored. All 3 of the last experiments reported indicate a strong diastatic action.

Potato-dextrose agar slants.—Growth good, white, glistening, not viscid but somewhat sticky, best at the base of the slant, noticeably flatter than on ordinary nutrient agar, margin even, somewhat spreading.

Milk.—By the sixth day, at a temperature of 25-30° C., there was no coagulation and no clearing. By the eighth day there was

a color change from light buff to warm buff (Ridgway). The medium was cleared slowly but there was no coagulation and by the end of 8 weeks most of the fluid was clear, clay-colored, with a somewhat slimy coagulum covering the bottom of the tube which, when agitated, broke up into lumps. At the end of 3 months most of the coagulum disappeared.

Litmus milk.—There was no change in color up to the fifteenth day when the medium began to turn blue at a temperature of 25-30° C. (original color a grayish blue). The behavior was otherwise the same as in plain milk.

Methylene blue in milk.—Reduction of the dye was prompt; in 24 hours the blue disappeared from the lower two-thirds of the medium leaving a blue rim above. By the end of 48 hours almost all of the blue had disappeared. Here also there was no curdling, indicating that no lab ferment (chymosin or rennin) was produced. This is further indicated by the fact that gelatin was not liquefied.

Grass decoction agars.—It seemed desirable to test the effect of different grass decoctions on growth, in particular the effect of grass decoction obtained from a susceptible host as compared with a decoction obtained from a resistant host. For this purpose 500 grams of washed, healthy leaves of *Chaetochloa lutescens* and of *C. viridis* were boiled in distilled water for 1 hour, the extract decanted, and the well-cooked leaves squeezed between cheesecloth in order to obtain a maximum amount of extract. Fifteen grams of agar were added and enough water to make one liter of each decoction. The growth on slants of both of these decoctions was meager and no difference was detected. The experiment indicates that between 2 different cooked decoctions, 1 of a resistant and the other of a susceptible plant, there was no difference in growth, and it is felt that further than this no conclusion seems justified.

Amino acids in relation to growth.—Time has not permitted a thorough study of the role of amino acids in the growth of the foxtail organism, but the experiment here reported is indicative of a line of work that should be fully investigated.

It should be explained that the amino acids were dissolved in distilled water, and in the case of tyrosine, hydrochloric acid was added in (1) and (2) to aid in dissolution, the amount of acid added being sufficient, as shown by the P_H obtained, to affect growth. The beef extract (Liebig's) was used in the same proportion as was used in nutrient broth, 3 grams per liter. The various solutions were sterilized in the autoclave at about 12 pounds pressure for 20 minutes. The experiment clearly indicates that the foxtail organism not only utilizes amino acids but that growth is influenced by the type of amino acid present, thus

TABLE VII

AMINO ACIDS IN RELATION TO GROWTH

Solution	P_H	Response
(1) 0.1% tyrosine*	4.6	No growth
(2) 0.1% tyrosine + 1.0% dextrose	4.6	No growth
(3) 0.1% tyrosine + beef extract	-------	Noticeable clouding
(4) 0.1% tyrosine + 1.0% dextrose + beef extract	-------	Heavy growth
(5) 0.1% leucine	6.8	Noticeable clouding
(6) 0.1% leucine + 1.0% dextrose	-------	Clouding more pronounced than in (5)
(7) 0.1% leucine + beef extract	-------	Better growth than in (6)
(8) 0.1% leucine + 1.0% dextrose + beef extract	-------	Growth as good as in (13)
(9) 0.1% leucine fraction (alanine, leucine, valine)	-------	Clouding more pronounced than in (5)
(10) 0.1% leucine fraction + 1% dextrose	-------	Marked clouding, better than (6) and (9)
(11) 0.1% leucine fraction + beef extract	-------	Growth about same as in (7)
(12) 0.1% leucine fraction + 1.0% dextrose + beef extract	-------	Growth somewhat superior to (13)
(13) nutrient broth (1.0% peptone + 3 grams beef extract)	-------	Good growth

*The amino acids utilized were kindly supplied by Dr. Barnett Sure.

the combination of alanine, leucine, and valine yields better growth than leucine alone, although the total amount of amino acid used was the same in both solutions. As compared with peptone (Difco) it is interesting to note that these amino acids in combination with dextrose and beef extract gave just as good a growth, and in case of the leucine fraction, the growth seemed heavier than in peptone media.

Indol production.—Both the sodium nitrite-sulphuric acid and the Ehrlich tests gave negative results at the end of the second, fourth and tenth days. The media used were Dunham's solution, nutrient broth, and one consisting of 1 per cent peptone, 0.5 per cent disodium phosphate and 0.1 per cent magnesium sulphate in a liter of distilled water. (*Bacillus Coli* gave a positive test in Dunham's solution at end of the fourth day, with Ehrlich's test.) It should be noted that according to the recent work of Norton and Sawyer (II, '21) it appears that the old tests, such as sodium nitrite-sulphuric acid, are not reliable.

Nitrite production.—Growth in nitrate broth was fairly good; within 48 hours there was a slight growth on the surface, a

marked clouding and a heavy viscid growth in the bottom. Tests for nitrite on the tenth day with starch water, potassium iodide, sulphuric acid gave a deep blue color, indicating the presence of nitrites (uninoculated tubes when tested gave no color).

Hydrogen sulphide production.—Tests with paper saturated with lead acetate hung over nutrient broth and over broth plus potato starch showed no signs of hydrogen sulphide production.

Ammonia production.—Tests with filter paper saturated with Nessler's reagent which was suspended over cultures in nutrient broth showed a reddish brown color, rapidly developing on the paper upon heating the solution, hence, that ammonia was produced.

Gas production.—One per cent of the following substances were used in nutrient broth kept at about 25° C. in fermentation tubes: dextrose, galactose, mannite, lactose, maltose, saccharose, inulin, and glycerin. Growth was very good in all and was sharply limited to the medium contained in the open arm, medium not being agitated (see pl. 7, fig. 1). No gas was produced in the closed arm in any of the substances listed in contrast to *Bacillus Coli* which showed abundant gas in a number of the solutions. This does not mean that no gas was produced; it has already been shown that ammonia was produced in nutrient broth, but that because of the growth being limited to the open arm, any gas which was produced, and which in this organism must not be noticeable, passed off into the atmosphere through the open arm. However, no carbon-dioxide or hydrogen has ever been collected and it is not likely that they were formed in any appreciable quantity in this organism since it is not an acid producer.

Acid production.—Various experiments indicate that this organism does not produce acid in any of the ordinary media. It has already been noted that in acid broths the medium changes toward neutrality or becomes alkaline and that litmus milk becomes blue. Likewise, in the presence of carbohydrates there is no acid production at any time during the growth period. The following experiments illustrate this. Litmus agars containing no other nutrients than 1 per cent of the following carbohydrates remained blue for 2 months, showing no pinkish tinge at any time: dextrose, galactose, mannite, lactose, saccharose, raffinose, and glycerin. Nutrient agar with brom cresolpurple as indicator containing 1 per cent of the following: dextrose, galactose, lactose, and saccharose showed no trace of yellow in 10 days' growth. Lactose agar stabs with Andrade-Penny's indicator (Holman, II, '14) showed no color, in contrast to *Bacillus Coli* which developed a conspicuous pink color in 48 hours. Instead of producing acid this organism produced alkali in the presence of carbohydrates as is shown in the following table. The media

consisted of nutrient broth plus 1 per cent each of the carbohydrates listed.

TABLE VIII

Type of carbohydrate	Original P_H	P_H at end of 5 days	P_H at end of 10 days
Dextrose	7.2	7.2	7.3
Levulose	7.2	7.2	7.4
Mannite	7.2	7.4	7.4
Lactose	7.2	8.0	8.2
Maltose	7.2	8.0	8.0
Saccharose	7.2	8.0	8.2
Glycerin	7.2	7.2	7.2
Broth (without carbohydrate)	7.2	8.0	8.0

Growth was good in all and exceptionally heavy in dextrose and levulose broth. In every case except glycerin the medium was actually rendered more alkaline instead of acid. Does this mean that the foxtail organism does not utilize carbohydrates? If the production of acid and gas was taken as the only indication of carbohydrate utilization (as some investigators seem to believe) the question would be answered in the negative; yet in the case of the foxtail organism there is evidence to indicate that carbohydrates were used without the production of acid or gas (ammonia production resulted from protein decomposition). It is well known that carbohydrate utilization in complex and in simple organisms commonly results in the production of acid and of gas, but it is also known that certain organisms, such as yeasts, decompose carbohydrates and instead of yielding acid they produce alcohol. There are also definite cases on record where carbohydrates are shown to have been used and alkali produced. Thus Miss Karrer (II, '21, p. 78) states: " . . . . the reaction of all the culture solutions with starch as the source of energy was changed during the growth of the fungus (*Fusarium* sp.), this shift being toward increased alkalinity." She presents numerous curves which show the amounts of starch utilized in nutrient media which were acid at the beginning of the experiment and which developed alkali as growth took place. It is to be noted further that in the nutrient media she employed there was no organic nitrogen.

It is not intended to go into a lengthy discussion of acid and alkali production in relation to carbohydrates, how carbohydrates in the presence of nitrogenous food may first be utilized and thus exercise a "sparing" action on proteins, or how acid and alkali may be formed simultaneously, or how a preponderance of alkali may be formed either because of a preferential utilization of acid radicles or because alkali-yielding substances, such as

proteins, are utilized to a greater extent. Considerable work has been done on the subject and much remains to be elucidated. Briefly summarized, the following experiments clearly indicate that carbohydrates are utilized by the foxtail organism; first, the marked diastatic action on starch in various media; second, the good growth obtained on media containing nothing but potato cylinders; third, the slight growth obtained in aqueous solutions containing only 1 per cent of each of these sugars, dextrose, galactose, mannite, and saccharose; fourth, the increased growth in solutions containing sugar over those in which it is absent, in the amino acid series which was previously presented.

Hydrogen-ion concentration in relation to growth.—It has already been reported that the concentration of hydrogen ions plays an important part in the inhibition or enhancement of growth of the foxtail organism. In addition to the experiment on acid broths, nutrient broth was rendered acid or alkaline by adding hydrochloric acid and sodium hydroxide aseptically after the broth had been sterilized. The following series was used: P_H 4.8, 5.0, 6.6, 7.2, 7.4, 7.6, 8.5, 8.6, and 9.8. There was good growth in P_H 6.6, 7.2, 7.4, and 7.6, and no growth in P_H 4.8, 5.0, 8.5, 8.6, and 9.8. This series in combination with the other studies indicate that the optimum reaction lies between P_H 6.0 and P_H 8.0, that the lower limiting reaction for growth is around P_H 5.0, varying somewhat with different media, and that the upper limit for growth to occur is somewhat below P_H 8.5.

NUTRIENTS IN RELATION TO PRECIPITATE PRODUCTION

Production of a colorless zone followed by a white discoloration has already been reported on nutrient agar and on whey agar when these gave a slightly acid reaction. It has also been reported that no precipitate was obtained on nutrient gelatin. In an effort to determine which material in the media contained the ingredient which gave rise to the precipitate a number of experiments were tried. Beginning with agar an attempt was made to substitute other plant materials for the algal product and the gums arabic and tragacanth were tried without success. The first of these when dissolved in water does not give a stiff medium even at very high concentrations, and the second is extremely difficult to dissolve in water. Attempts were then made to thoroughly wash the agar according to the method used by Ayers, Mudge, and Rupp (II, '20). These authors found that unwashed agar contained various calcium and magnesium salts as well as proteins and that by a thorough washing considerable part of these impurities were removed. Shredded agar was used, and after washing the nutrients peptone and beef extract were added in the usual amounts; part of the medium was allowed to remain slightly acid, P_H 6.6, and part was rendered slightly alkaline with sodium hydroxide, P_H 7.2. Good growth was obtained

on both, and while no precipitate was obtained in the alkaline medium the characteristic white discoloration occurred on the acid medium, indicating that impurities contained in the agar were probably not responsible for the formation of precipitate. As agar itself is not ordinarily considered a food, it seems reasonable to conclude that agar was not responsible for the white precipitate.

Water was the next ingredient investigated. Previous tests having indicated that a medium containing tap water, when slightly acid in reaction, gave a heavier precipitate than distilled water, tap water was used. Tap water was therefore treated with strong hydrochloric acid in order to remove carbonates, and boiled for several hours. It was then incorporated into nutrient agar, and part was rendered alkaline, P_H 7.2, and another part acid, P_H 6.6; nutrient agar containing untreated tap water and also 1 part made alkaline and another acid were run at the same time as checks. Growth on the alkaline medium containing treated water was as good as on the medium containing untreated water, but the medium testing P_H 6.6 with treated water did not give as good growth as the slightly alkaline medium. White precipitate occurred in the acid media, both with treated and untreated water. This indicates that water does not influence the production of precipitate. (Chemical tests made of the water at Fayetteville, Ark., by the department of chemistry of the University of Arkansas indicate that of the materials found in the local tap water, carbonates made up by far the largest proportion, other substances being but slightly more than a trace.)

Substitutions were then attempted for peptone; the media used were (1) 1 per cent dextrose-beef extract agar; (2) 1 per cent lactose-beef extract agar; (3) 1 per cent dextrose, and 0.1 per cent tyrosine-beef extract agar. In all of these the ordinary 0.3 per cent beef extract was used, each divided into 2 parts, 1 rendered slightly acid and the other slightly alkaline. Growth in the first 2 was not as good as in media containing peptone, but the 1 containing tyrosine gave a growth which appeared fully as good as on ordinary nutrient agar. (Davis (II, '17) has found that the value of peptone is governed by the amino acids present and he has concluded that tyrosine and tryptophane are important constituents of satisfactory peptone.) On each of the 3 media listed, precipitate was obtained in the part testing slightly acid. This clearly indicates that the commercial peptone is not responsible for the white precipitate.

Beef extract is the remaining nutrient to be considered. Media containing but 1 per cent dextrose and 0.1 per cent tyrosine in agar were tried, and no precipitate was obtained either in acid or in alkaline media. Growth in these was not good, but this is not the factor in preventing precipitate production since the precipitate was produced in 1 per cent dextrose plus beef ex-

tract even though growth was poor. It may then be concluded that beef extract is the probable source of the white precipitate, and while no similar study has been made on whey agar it is quite probable that whey is the source of the precipitate in this latter medium.

CHEMICAL NATURE OF THE PRECIPITATE

The precipitate was soluble in all of the acids tried, including the following: glacial acetic, chromic, citric, hydrochloric, nitric, sulphuric, lactic, picric, and warm boric; it was insoluble in warm or cold water or organic solvents, including methyl, ethyl, and butyl alcohol, glycerin, acetone, ether, petroleum ether, chloroform, oil of turpentine, benzole, xylol, tuluol, benzine, carbon bisulphide, carbon tetrachloride, potassium permanganate, hydrogen peroxide, and ammonium peroxide. This indicates that the precipitate is an inorganic salt. Tests were made for carbonates and phosphates with the result that phosphates were clearly detected. Since the precipitate, while conspicuous, may be had in comparatively very small amounts it is rather difficult to test. The procedure was as follows: With a platinum loop the bacterial smear was carefully removed (all tests were made on agar slants); the tube was washed several times with distilled water and care was taken to see that all bacterial growth, where this was a possible factor, as well as particles of medium, were removed. One per cent solution of nitric acid was then used to dissolve the precipitate, the fluid decanted, filtered through fine filter-paper, and tested with ammonium molybdate reagent (see Treadwell's 'Qualitative Analysis'). Tubes containing the same media but without showing any precipitate, the ones reacting alkaline, were treated in exactly similar manner and run at the same time. Many such tests show that the precipitate, if not wholly, is in large part phosphate, as indicated by the yellow color, and precipitate developed in solutions coming from tubes which originally contained the white discoloration.

It is very difficult to obtain a test of any nutrient agar in which the yellow precipitate produced by ammonium molybdate is entirely absent, since the addition of strong acid may not only disintegrate any bacteria which may be present but may also dissolve some of the nutrient medium. However, there is no difficulty in obtaining a marked quantitative difference of yellow precipitate when the same amount of reagents are used in tubes possessing the white precipitate as against tubes which do not, those tubes containing the precipitate always giving a greater amount of yellow ammonium phosphomolybdate. In a number of tests, where the bacterial growth was removed *in toto*, often very difficult to do without breaking up the medium, and where the acid was allowed to act only a short time, no yellow

precipitate occurred in tubes which did not possess the white precipitate.

It seems to be well established that beef extract contains phosphates, the following statement in Eyre's 'Bacteriological Technique,' p. 128, indicating this: "Meat extract . . . is acid in its reaction owing to the presence of acid phosphates . . ." The following appears to be a plausible explanation for the production of a colorless zone plus a white precipitate on acid media. It has been shown that the foxtail organism in its growth on various media, including nutrient agar, produces alkali. The alkali acts on the acid phosphates and causes them to precipitate out of solution. (It is well known that various acid phosphates are precipitated out of solution when alkali is added.) Immediately around the organism the phosphate is probably used up as growth proceeds, while the phosphate beyond a certain distance of the bacterial growth, not having been used, is acted on by the alkali which diffuses from the region of growth and is precipitated out of solution. There is also the possibility of an action by a carbon dioxide gradient which, being greatest in the region immediately surrounding the colony, might prevent precipitation by the acid reaction. No precipitate is produced on alkaline media, probably because the substances from which the white precipitate is derived were largely precipitated out when the medium was rendered alkaline. This seems to be in keeping with Eyre's observation (p. 150). In giving directions for making nutrient agar he recommends the addition of sodium hydroxide until a reaction of +10 is obtained (this often is equivalent to P_H 7.0 or P_H 7.2 as previously shown) and further recommends the following as the next step after the addition of alkali. "Replace in the steamer for twenty minutes (to complete the precipitation of the phosphates, etc.)."

Which phosphates are involved in this precipitate has not been determined. In experimenting with anhydrous disodium glycerophosphate Mellon and his associates (II, '21) decided that this substance acts as a solvent for calcium and magnesium salts. Accordingly, it was incorporated into nutrient agar in the proportion they recommend. The medium was divided into several parts, some being rendered acid and some alkaline by the addition of hydrochloric acid or by sodium hydroxide giving the following reactions: P_H 6.0, 6.3, 6.6, 6.8, and 7.4. Growth on this medium as a whole is somewhat better than on ordinary nutrient agar. It makes a clear medium although a dense precipitate settles at the bottom of the tubes after sterilization. No white precipitate was produced on either the acid or alkaline side. It is of course difficult to say how this is brought about, whether the presence of this salt prevents precipitation by its solvent action on calcium and magnesium salts, or because this salt when added to peptone-beef extract agar precipitates out ma-

terials which would otherwise enter into the production of white precipitate, or because of its buffer action in preventing a change in the hydrogen-ion concentration. At any rate, it is quite probable that the white precipitate is in the nature of a complex phosphate and not a simple calcium or magnesium salt.

PHYSIOLOGICAL REACTIONS

Effect of temperature on growth.—The medium used was nutrient broth testing P_H 6.8, care being taken to add approximately the same amount of inoculum, 0.1 cc, of 48-hour broth culture, to each 10 cc. of medium. The organism was subjected to the following temperatures (degrees centigrade): 10, 14, 20, 25, 30, 35. 40 . With the exception of the 30° incubator which maintained very nearly a constant temperature, there was some variation in all of the others amounting to 1–2 degrees, so that the temperatures given are not absolute. In 24 hours there was a heavy clouding at 34–35°, almost as heavy at 30°, noticeably less at 25 , only a slight growth in the upper fourth of the broth at 20°, no visible growth at 14°, and none at 10°. In 48 hours a slight growth was visible at 14°. At the end of a week there was a faint clouding at 10°, more noticeable clouding at 14°, and beginning with 20° growth was very marked up to 35°, with none at 40°. Growth appeared first and was heaviest at 30–35°, which may be regarded as the optimum zone. The minimum zone has not been determined but it is probably around 0°, while the maximum is around 40°. Death occurred at 41 to 43°. It is thus seen that the optimum, a rather high temperature, is quite close to the maximum which in turn is close to the thermal death point.

Effect of drying.—Bacterial smears on sterile cover glasses were kept dry for 1 hour, 5 hours, 48 hours, and 3 days, at 25° C. When these glasses were dropped into broth growth occurred in all except the one kept dry for 3 days. One would conclude then from this experiment that the foxtail organism is sensitive to desiccation. The following data indicate that such a conclusion is quite false. Diseased material collected July 30, 1921, and kept in a dry condition in the laboratory at about 25° C. until March 24, 1922, a period of about 7 months, was surface sterilized in the ordinary manner, macerated in sterile water, and sprayed on oat seedlings, giving abundant infections, while a check pot sprayed with sterile water showed none. The experiment was duplicated with the same results.

The writer is convinced that the effect of drying cannot be measured merely by smears on glass, and taken by themselves such tests are apt to lead to erroneous conclusions. The reason for this is apparent, bacteria within infected host tissue being of course more or less closely associated with cellular matter.

The action of plant colloids in taking up and holding water has been carefully investigated (see MacDougal, Carnegie Inst. Publ. 297) so that the colloids in association with bacteria must be considered in any water relationships. It thus appears that the foxtail organism is resistant to drying.

Effect of freezing.—Using 48-hour broth cultures, 0.2 cc. was inoculated in tubes containing 10 cc. of broth. Two tubes were placed into a freezing, brine-ice mixture, and 2 into an incubator at 35° C. After 2 hours the 2 tubes, which had remained in the form of solid frozen masses for more than 1½ hours, were removed from the freezing mixture, thawed out, and poured plates made from calculated dilutions. Check tubes kept in the incubator for the same length of time were treated in the same way. Using equal amounts of inoculum no noticeable difference in the number of colonies which developed was obtained. The experiment indicates that the foxtail organism is not readily killed by freezing and thawing.

Effect of sunlight.—Moderately thick sowings of a 48-hour broth culture were made on nutrient agar-poured plates. One-half of each plate was covered with black paper and the plates, resting on ice, were exposed to a bright December sun for 5, 10, 15, and 20 minutes, triplicates being used for each time period. They were then incubated at about 32° C. No difference in number of colonies could be detected for any of the periods in the exposed as against the covered half. The results were rather unexpected since bacterial pathogens are often described as sensitive to sunlight, certainly within the range employed, 5 to 15 minutes being sufficient to kill or very much retard colony development in a large number of forms. The experiment was therefore duplicated in May and exposures of 15, 30, and 45 minutes made to a bright sun. There was hardly more than a 10 per cent reduction in the number of colonies on plates exposed 30 and 45 minutes as compared to those exposed 15 minutes, the difference being within the limits of experimental error. The number of colonies (about 150) which developed after 45 minutes on the exposed half as compared to the covered half was hardly different. The organism does not appear to be very sensitive to sunlight.

Overwintering.—Experiments on the effect of desiccation and freezing indicate that the organism has no difficulty in overwintering. Unfortunately diseased material which had been placed out of doors in the fall to be tested the following spring was destroyed so that there is no direct evidence for overwintering. Field observations as well as laboratory studies indicate that overwintering is readily accomplished. The writer has already recorded the presence of the disease for 4 successive seasons on the same field. The organism may be carried over winter either

in diseased glumes, or possibly in the soil in diseased leaves.

Vitality on culture media.—If the substratum is not permitted to dry down, the foxtail organism will continue to live for a long time. In liquid media, such as nutrient broth and milk, growth may be obtained after a period of 6 months, and infections are readily produced from broth cultures kept 4 months at about 25° C. However, when the organism growing on nutrient agar slants is kept in the ice-box for 9 months it loses its viability.

Group number.—According to the most recent chart adopted by the Society of American Bacteriologists (Conn, et al, II, '20), the foxtail organism has the following index number: 5322-31220-1333.

Technical Description

Pseudomonas alboprecipitans,[1] n. sp.[2]

Narrow rods with rounded ends, solitary or in pairs; average measurement of single rods 0.6 by 1.8 μ, motile by a single polar flagellum; no spores, zoogloea or irregular forms; capsules present; strict aerobe; surface colonies on nutrient agar white, round, somewhat raised, smooth, amorphous, sticky, margins entire, surrounded by colorless areas followed by a white precipitate on media testing acid, as P_H 6.6; nitrates reduced to nitrites; ammonia produced; indol and hydrogen sulphide not produced; no acid or gas produced in the presence or absence of carbohydrates; diastatic action strong; no growth in Cohn's solution but growth in Uschinsky's and Fermi's solutions is fair; minimum temperature about 0° C., optimum between 30 and 35°, maximum about 40°, thermal death point 41 43°; vitality on culture media comparatively long; not sensitive to drying or freezing; not very sensitive to sunlight; Gram-negative, not acid fast. Pathogenic to *Chaetochloa lutescens, C. geniculata, C. italica, Avena sativa, Holcus Sorghum, H. Sorghum sudanensis, Hordeum vulgare, Secale cereale, Triticum satirum*, and *Zea Mays.*

Summary

The bacterial disease here described is common on yellow foxtail in Arkansas. No concerted effort has been made to discover the disease on other grasses but artificial inoculations show that the pathogen is infectious on wheat, oats, rye, barley, corn, Sudan grass, millet, and perennial foxtail.

[1]According to the classification suggested by Smith (II, '05, p. 171) the combination would be *Bacterium alboprecipitans* n. sp.

[2]**Pseudomonas alboprecipitans,** sp. nov., aerobus; baculis asporis cylindricis singulis vel binatis apicibus utrinque rotundatis flagello uno polare mobile, baculis solitariis 0.6×1.8 μ.

Coloniae in agar-agar rotundatae albae leves, marginibus plenis; in culturis acidi minimi zonae hyalinae factae sunt secutae a precipito albo. Gelatina non liquefacit. Acidum et gas non efficiuntur.

Habitat in foliis vivis *Chaetochloae lutescentis, C. italicae, C. geniculatae, Tritici vulgaris, Secalis cerealis, Avenae sativae, Hordei vulgaris, Zeae Mays, Holci Sorghi.* Arkansas, Amer. bor.

A large number of varieties of various cereals are subject to attack. Artificial inoculations also indicate that the organism is capable of doing serious damage, particularly to seedlings of oats and barley.

Lesions on foxtail appear as light brown or dark brown spots and streaks of no definite size or shape and may occur on any part above ground, but are most often found on blades and sheaths.

Attacks on other hosts vary from light yellow indefinite areas, often with a reddish tinge in the case of oats, to grayish-green, markedly withered areas.

The disease appears to be different from any other known bacterial disease of grasses.

Attacked tissues teem with bacteria which discolor, disintegrate and finally kill the invaded tissue. Entrance to the host is by means of stomata and water pores.

The organism is a single-flagellate rod, white in culture, with colonies surrounded by a characteristic colorless area followed by a white precipitate on slightly acid media. It is described as *Pseudomonas alboprecipitans* n. sp.

The meaning of hydrogen-ion concentration, its relation to titratable acidity, methods of measuring it, and the necessity of utilizing it in the study of bacterial pathogens are discussed. Comparisons are given between Fuller's scale and P_H values.

Numerous cultural reactions are presented, including a method for definitely controlling precipitate production by means of varying the hydrogen-ion concentration; the relationship of various organic anions to growth, comparison between cotton and glass wool as filtering agents, the use of lima bean agar in bacterial studies, and a study of several amino acids in relation to growth of bacteria are also discussed.

It is found that beef extract is the probable source of the white precipitate in media which contain the extract and that this precipitate is a phosphate.

Part of the work presented in this paper was prosecuted as an Adams project at the Agricultural Experiment Station of the University of Arkansas. Special thanks are due to Dean Bradford Knapp for facilities made available and to Dr. J. A. Elliott who made the colored drawings and who gave kindly assistance and encouragement in the prosecution of the work. The work was continued at the Missouri Botanical Garden under the guidance of Dr. B. M. Duggar, and I am greatly indebted to him for very helpful suggestions and for critical advice. Thanks are also due to Dr. George T. Moore for the privileges and facilities of the Missouri Botanical Garden.

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Explanation of Plate

PLATE 1

Fig. 1. Natural infections on foxtail (3 leaves).
Fig. 2. Artificial infections on foxtail (2 leaves).
Fig. 3. Artificial infections on sorghum, variety "Amber Cane".
Fig. 4. Artificial infections on *Chaetochloa geniculata*.
Fig. 5. Artificial infections on corn.

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Explanation of Plate

PLATE 2

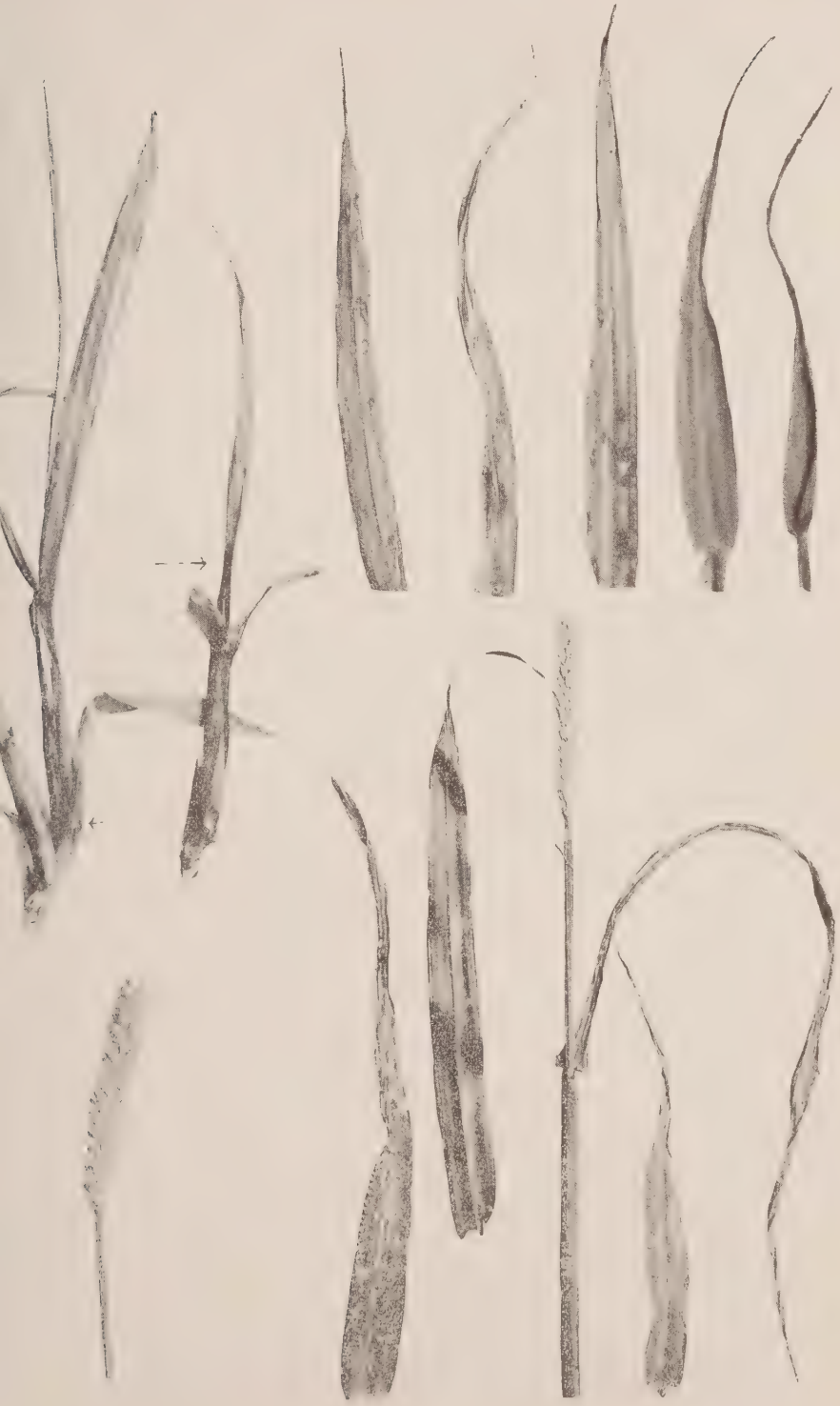
Fig. 1. Artificial infections on barley (3 leaves).
Fig. 2. Artificial infections on oats (6 leaves).
Fig. 3. Artificial infections on wheat (4 leaves).
Fig. 4. Artificial infections on rye.

Explanation of Plate

PLATE 3

Upper figures represent various types of natural infections on foxtail, including blackish streaks and blotches, small dark brown, oval-shaped or roundish spots with light centers and withered light brown tips.

Lower right-hand figures represent artificial infections; left-hand figure a normal head of foxtail *(Chaetochloa lutescens)*.



Explanation of Plate

PLATE 4

Fig. 1. Pot on the left—dead oat plants 4 days after inoculation with the foxtail organism; on the right—check plants of the same age sprayed with sterile water.

Fig. 2. On the right—badly diseased barley seedlings 4 days after inoculation; on the left—barley plants of the same age sprayed with sterile water.

Explanation of Plate

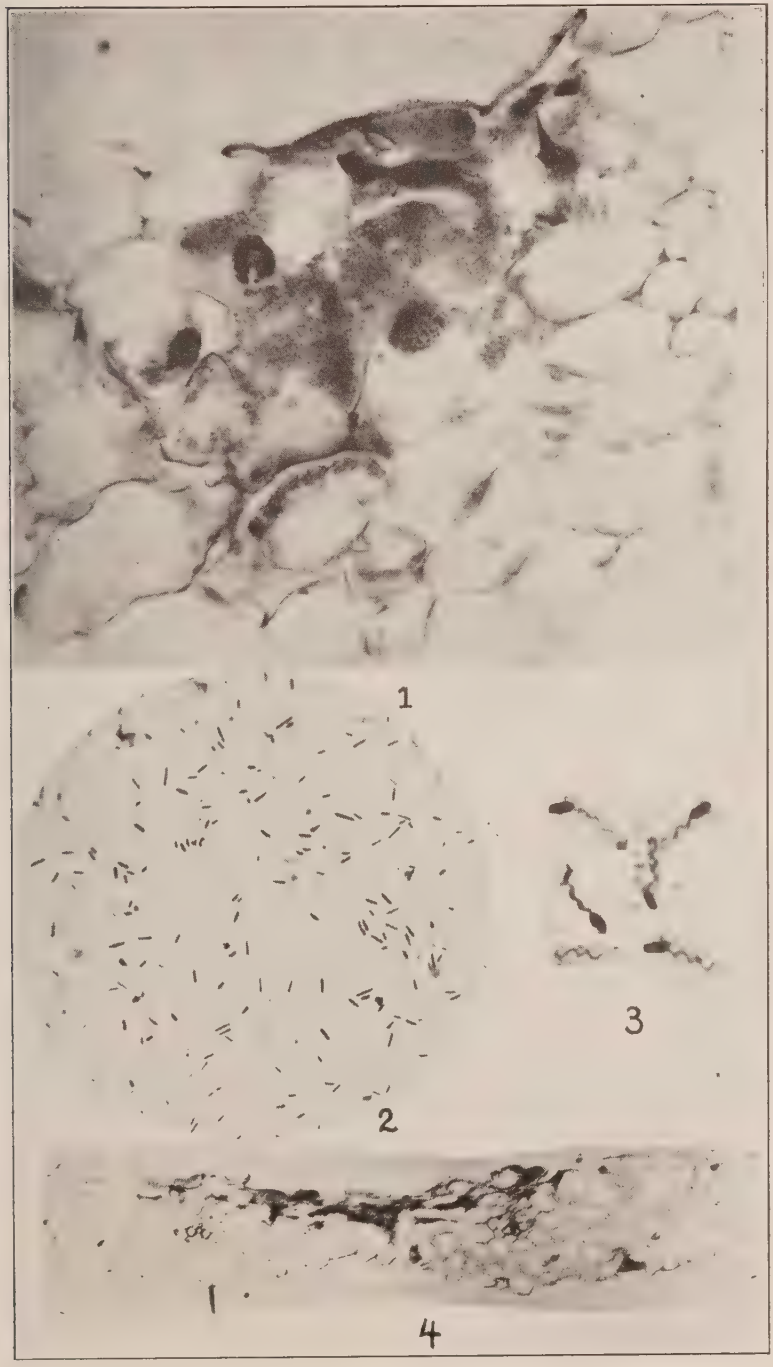
PLATE 5

Fig. 1. Section through a diseased spot of an oat leaf artificially infected, showing portion of a substomatal cavity as well as neighboring cells and intercellular spaces filled with bacteria. Magnified about 950 times.

Fig. 2. Foxtail organism stained with carbol fuchsin, obtained from young agar slant culture. Magnified about 1000 times.

Fig. 3. Foxtail organism stained with Van Ermengem's flagella stain. Magnified about 1200 times. Bodies of the organisms, except the lower ones to the left, retouched; all flagella remain untouched.

Fig. 4. Cross-section of a diseased oat spot showing discoloration and collapse of attacked cells. Magnified about 450 times.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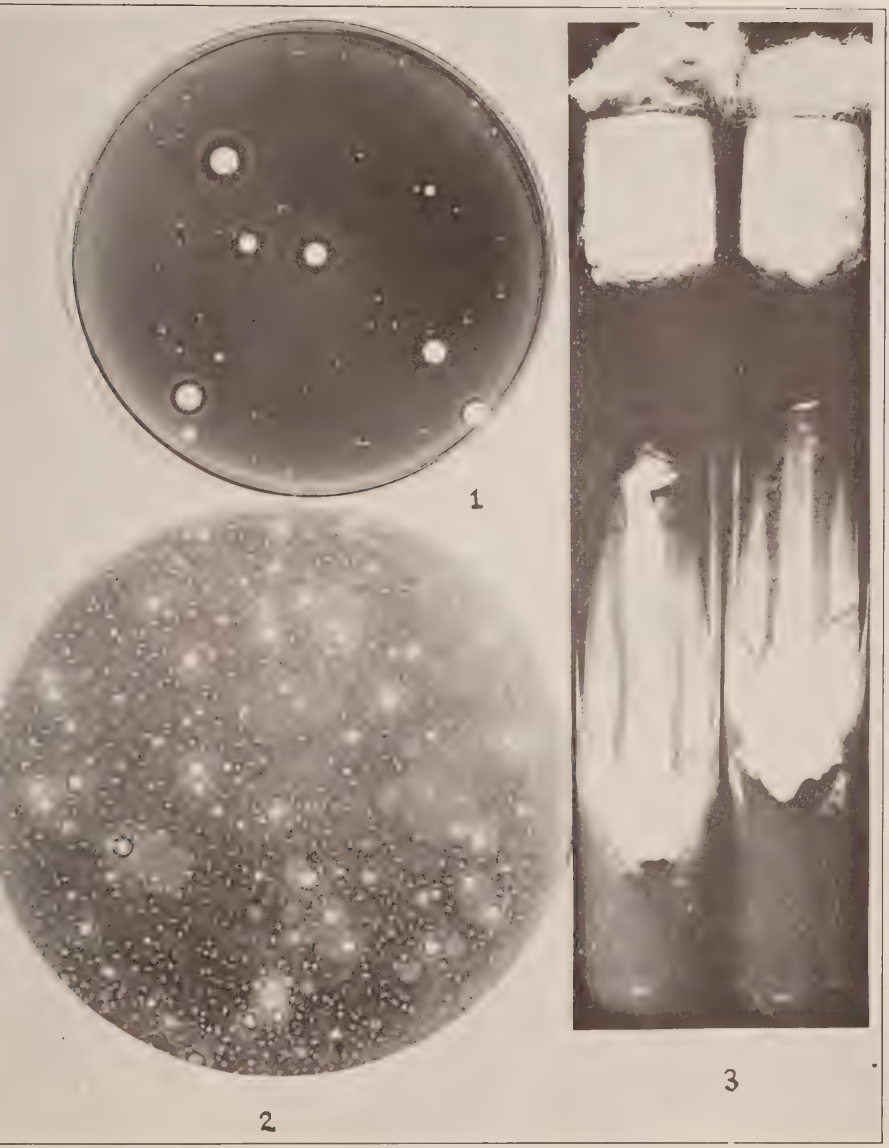
Explanation of Plate

PLATE 6

Fig. 1. Four-day colonies on nutrient agar testing P_H 6.6, showing colorless zone surrounded by a marked white precipitate.

Fig. 2. Same as fig. 1, showing a thickly sown plate with a coalescing and spreading grayish sheet of colonies deeply imbedded and growing next to the glass.

Fig. 3. Ten-day slant of nutrient agar testing P_H 6.4 showing colorless zone surrounded by a marked white precipitate.



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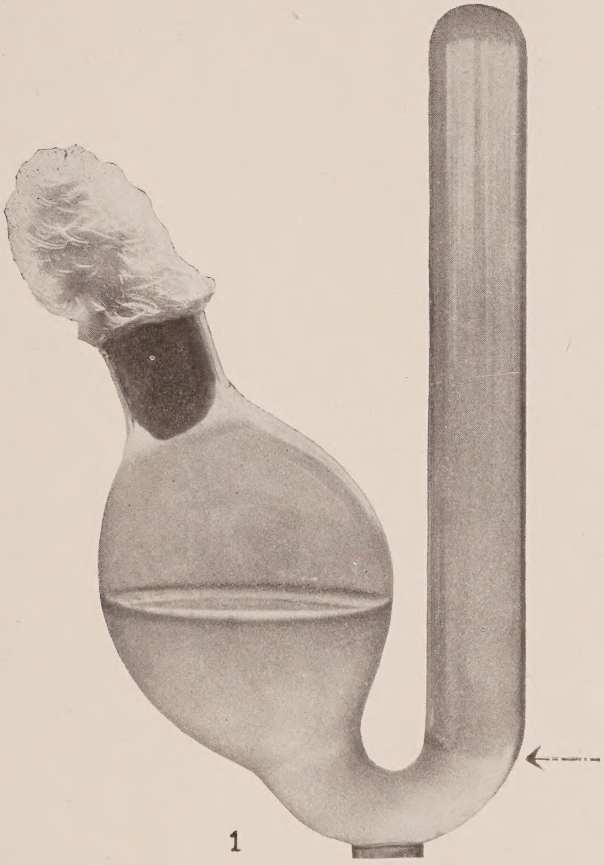
Explanation of Plate

PLATE 7

Fig. 1. Dense clouding in open arm in 1 per cent mannite-nutrient broth with no growth above the arrow point, and no gas.

Fig. 2. Wheat leaves inoculated at infected points with a pure culture of the foxtail organism, by means of a sterilized platinum loop.



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